Prodrugs activated by reactive oxygen species for use in the treatment of inflammatory diseases and cancer

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(54) Title: PRODRUGS ACTIVATED BY REACTIVE OXYGEN SPECIES FOR USE IN THE TREATMENT OF INFLAMMATORY DISEASES AND CANCER

(57) Abstract: Prodrugs activated predominantly or exclusively in inflammatory tissue, more particularly prodrugs of methotrexate and derivatives thereof, which are selectively activated by Reactive Oxygen Species (ROS) in inflammatory tissues associated with cancer and inflammatory diseases, as well as method for preparing said prodrugs.

PRODRUGS ACTIVATED BY REACTIVE OXYGEN SPECIES FOR USE IN THE TREATMENT OF INFLAMMATORY DISEASES AND CANCER

FIELD OF THE INVENTION

The present invention relates to prodrugs which are activated predominantly or exclusively in inflammatory tissue. More particularly, the present invention relates to prodrugs of methotrexate and derivatives thereof, which are selectively activated by Reactive Oxygen Species (ROS) in inflammatory tissues associated with cancer and inflammatory diseases such as rheumatoid arthritis (RA), juvenile dermatomyositis, psoriasis, psoriatic arthritis, lupus, sarcoidosis, Crohn's disease, colitis ulcerosa, multiple sclerosis, Amyotropic Lateral Sclerosis (ALS), atopic dermatitis, eczema etc.

BACKGROUND OF THE INVENTION

The current therapies for the treatment of cancer and the palliation of symptoms in chronic inflammatory diseases such as rheumatoid arthritis (RA), employing chemotherapy and anti-inflammatory therapeutics, are well-known to produce severe side-effects due their side-effect profile and poor selectivity.

Prodrugs are masked forms of pharmacologically active agents designed to undergo *in vivo* activation by specific stimuli. By introduction of prodrug chemical moieties that makes the drug in question inactive in healthy tissue and selectively activated in diseased tissue the side-effect profile and the selectivity may be improved significantly.

It is well-known that the concentration of Reactive Oxygen Species (ROS) is increased in inflammatory tissues associated with cancer and rheumatoid arthritis compared to healthy tissue. This unique environment of the inflammatory tissue can therefore be used as a trigger stimulus and in turn enables more selective palliative treatment of diseases associated with chronic inflammation, as well as in cancer therapy, by reducing side-effects stemming from cross-reactivity with healthy tissue.

Methotrexate is a well-known anti-cancer drug, a so-called anti-folate acting by inhibiting the metabolism of folic acid via dihydrofolate reductase. Methotrexate is also widely used as a disease-modifying treatment for some autoimmune diseases, including rheumatoid arthritis, juvenile dermatomyositis, psoriasis, psoriatic arthritis, lupus, sarcoidosis, and Crohn's disease.

US 2015/0005352 A1 discloses ROS-sensitive prodrug compositions and methods of treating ROS-associated diseases by administering the ROS-sensitive prodrug compositions.

WO 2012/123076 A1 relates to ferrocene-based compounds and their use as ROS-regulating prodrugs.

Xiaohua Peng & Varsha Gandhi, "ROS-activated anticancer prodrugs: a new strategy for tumor-specific damage", Therapeutic Delivery 2012, 3, 823-833 discloses the use of boronic acids/esters as triggers for developing ROS-activated anticancer prodrugs that target cancer cells.

US 2013/0045949 A1 related to prodrugs that are selectively activated to produce active anti-cancer agents in tumor cells using phenylboronates and phenylboronic acids as the trigger moiety.

Yunyan Kuang, Kumudha Balakrishnan, Varsha Gandhi, and Xiaohua Peng, "Hydrogen peroxide inducible DNA cross-linking agents: targeted anticancer prodrugs", J. Am. Chem. Soc. 2011, 133, 19278-19281 discloses a series of 3 phenyl boronic acid- and boronate-based compounds used as hydrogen peroxide sensitive prodrugs for the treatment of cancer.

WEI WEN-HAO ET AL.: "Gadolinium texaphyrin-methotrexate conjugates. Towards improved cancer chemotherapeutic agents", ORGANIC & BIOMOLECULAR CHEMISTRY, ROYAL SOCIETY OF CHEMISTRY, GB, vol. 3, no. 18, 21 September 2005, p. 3290-3296 discloses methotrexate conjugates and their use.

ACHILLI, C. ET AL.: "Folic acid-conjugated 4-Amino-Phenylboronate, a Boron-Containing Compound Designed for Boron Neutron Capture Therapy, is an Unexpected Agonist for Human Neutrophils and Platelets", CHEM BIO DRUG DES, vol. 83, 2013, p. 532-540 discloses folic acid-conjugated 4-amino-phenylboronate as a possible compound for the selective delivery of ^{10}B in Boron Neutron Capture Therapy (BNCT).

ROSOWSKY A ET AL.: "SYNTHESIS OF BIOLOGICAL ACTIVITY OF METHOTREXATE ANALOGUES WITH TWO ACID GROUPS AND A HYDROPHOBIC AROMATIC RING IN THE SIDE CHAIN", JOURNAL OF MEDICINAL CHEMISTRY AMERICAN CHEMICAL SOCIETY, US, vol. 34, no. 2, 1 January 1991, p. 574-579 discloses γ-(m-carboxyanilide) and γ-(m-boronoanilide) deivatives of methotrexate and γ-(m-carboxyanilide) derivatives of aminopterin.

KHAN; Z.A. ET AL.: "Methotrexate: a detailed review on drug delivery and clinical spects", EXPERT OPINION ON DRUG DELIVERY, vol. 9, 2012, p. 151-169 describes methotrexate and uses thereof

for the treatment of various types of malignancy, psoriasis, rheumatological diseases and the medical termination of pregnancy.

US 2013/045949 A1 discloses compounds that may be selectively activated to produce active anti-cancer agents in tumor cells.

US 2014/0378673 A1 relates to hypoxia selective prodrugs.

There is still a need for novel prodrugs of ROS-sensitive drug compositions, in particular prodrugs of methotrexate, which may be used for site-specific treatment, are stable and lend themselves for up-scaling.

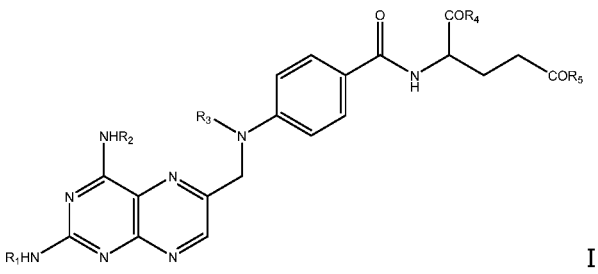
OBJECT OF THE INVENTION

It is an object of embodiments of the invention to provide prodrugs of ROS-sensitive drug compositions, in particular prodrugs of methotrexate, which are selectively activated in inflammatory tissues, have a beneficial cytotoxicity in target cells, low (no) cytotoxicity in healthy cells, are stable, and have a satisfactory bioavailability at the intended site of action.

SUMMARY OF THE INVENTION

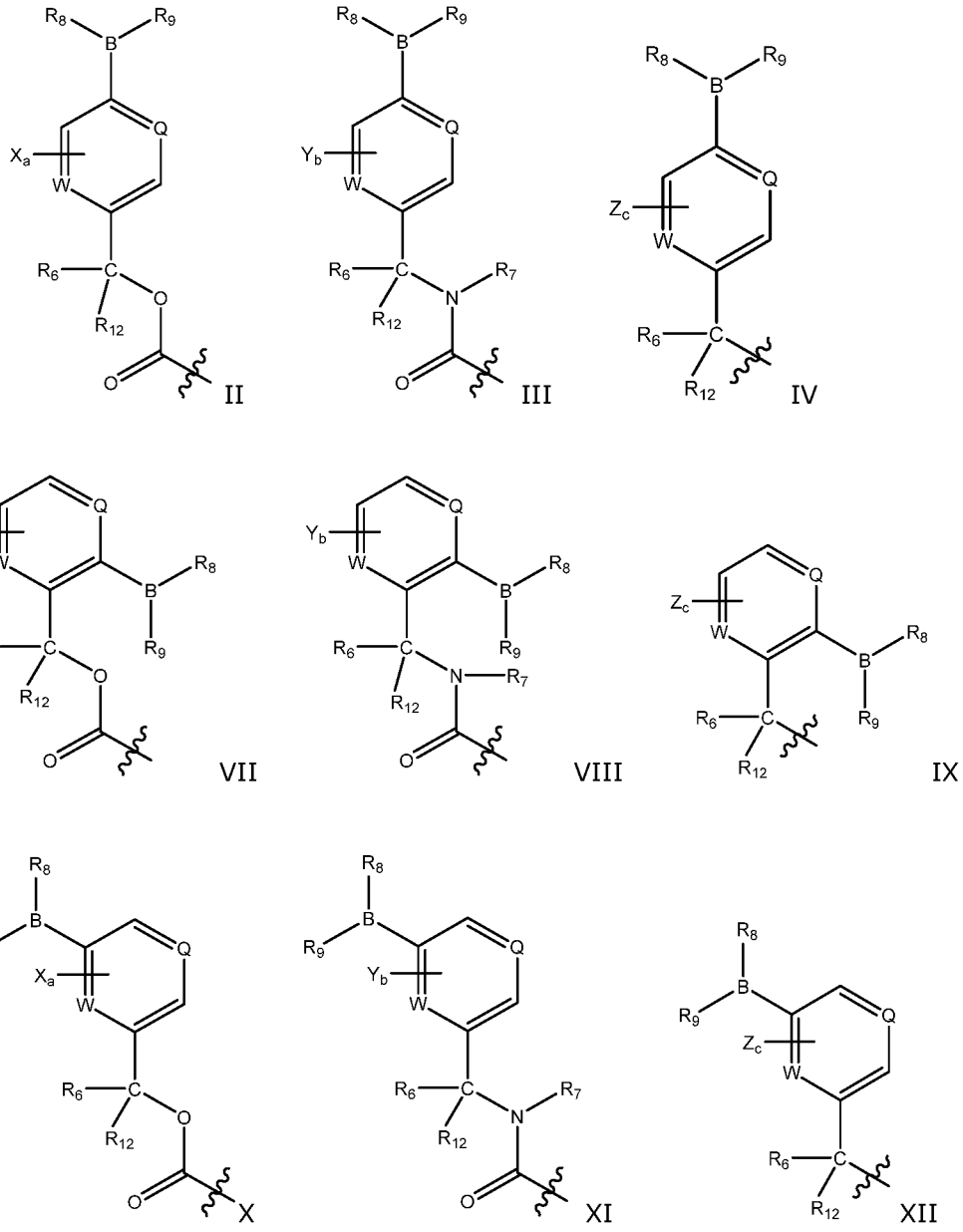
It has been found by the present inventor(s) that the below disclosed derivatives of methotrexate of formula I are ROS-sensitive and are selectively activated in inflammatory tissues and thus lend themselves for site-specific treatment with methotrexate.

So, in a first aspect the present invention relates to a compound of the formula I



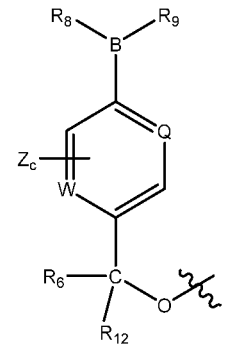
I

wherein R1 and R2 are independently selected from the group consisting of hydrogen and a moiety of the formula II, III, IV, VII, VIII, IX, X, XI, or XII

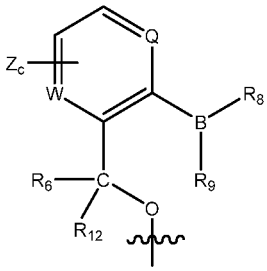


R3 is selected from the group consisting of hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-4}alkynyl, C_{6-12}aryl, C_{4-11} heteroaryl and a moiety of the formula II, III, IV, VII, VIII, IX, X, XI, or XII above;

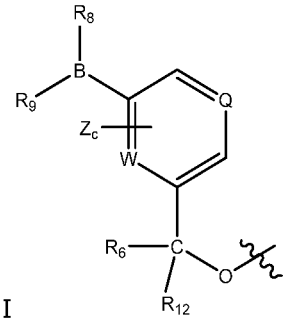
R4 and R5 are independently selected from the group consisting of OH, O-C_{1-6}alkyl, O-C_{2-6}alkenyl, O-C_{6-12}aryl, O-C_{4-11}heteroaryl and a moiety of the formula V, VI, XIII, XIV, XV, XVI, or XVII;



V



XIII



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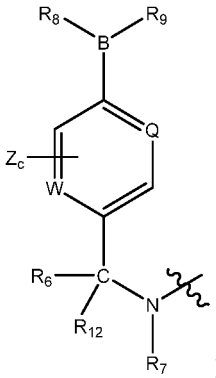

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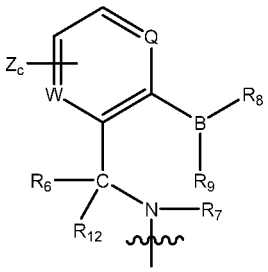

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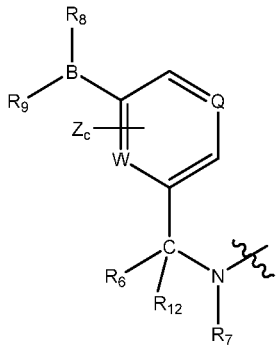

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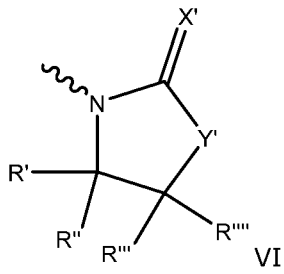


VI

R6, R7 and R12 are independently selected from the group consisting of hydrogen, CF_3, C_{1-6}alkyl, C_{2-6}alkenyl, C_{6-12}aryl, and C_{4-11}heteroaryl;

R8 and R9 are independently hydroxyl groups or R8 and R9 form, together with the intervening B and O atoms, a pinacol, catechol, diethanolamine, N-methyldiethanolamine or N-methyliminodiacetic acid (MIDA) boronate group;

W and Q are independently C or N;

wherein each of X, Y and Z are selected from the group consisting of halogen, amino, nitro, cyano, hydroxyl, CF_3, C_{1-6}alkyl, C_{1-6}alkoxy, C_{2-6}alkenyl, C_{2-6}alkenyloxy, C_{6-12}aryl, C_{4-11}heteroaryl; wherein each of said C_{1-6}alkyl, C_{2-6}alkenyl, C_{6-12}aryl, C_{4-11}heteroaryl may be substituted by one or more

substituents selected from the group consisting of halogen, amino, nitro, cyano, hydroxyl, CF_3, and C_{1-6}alkyl; and

each of a, b and c are integers in the range 0-4;

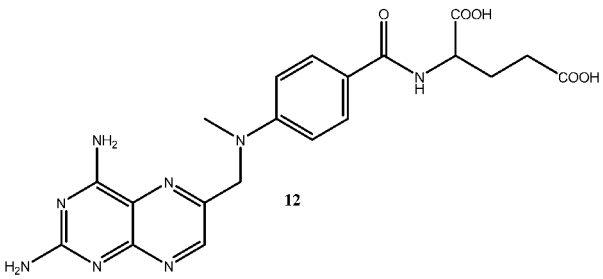
X′ and Y′ are independently S or O, and R′, R″, R‴ and R⁗ are independently selected from hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{6-12}aryl, C_{4-11}heteroaryl, and C_{1-6}alkyl-C_{6-12}aryl;

wherein, if each of R1, R2 and R3 are different from a moiety selected from a moiety of the formula II, III, IV, VII, VIII, IX, X, XI, or XII then at least one of R4 and R5 is a moiety of the formula V, XIII, XIV, XV, XVI, or XVII;

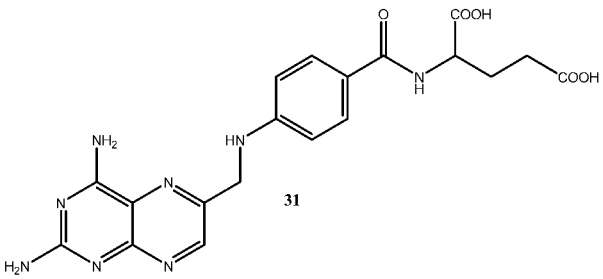
as well as pharmaceutically acceptable salts, solvates, and stereoisomers thereof.

In a second aspect the present invention relates to a method for the preparation of a compound according to the invention, comprising the steps:

a) Providing methotrexate (MTX) of the formula **12** or aminopterin (AMT) of the formula **31**



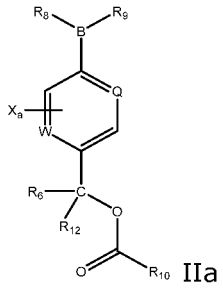
12

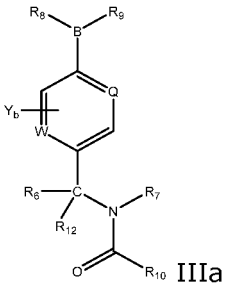


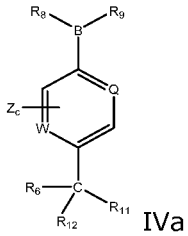
31

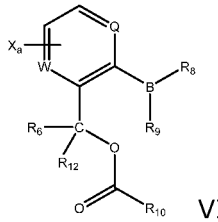
or any protected versions of them

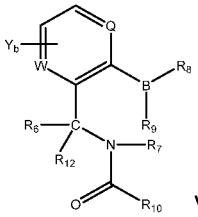
b) Providing a compound of formula IIa, IIIa, IVa, VIa, VIIa, VIIIa, IXa, Xa, XIa, XIIa, XVIIIa, XIXa, XXa, XXIa, XXIIa, or XXIIIa

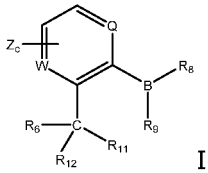

IIa


IIIa


IVa


VIIa


VIIIa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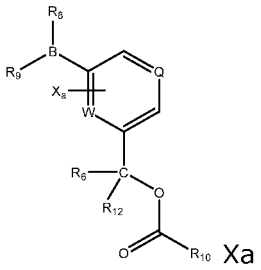
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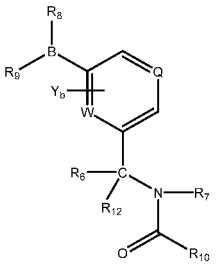
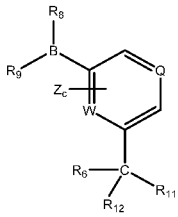

XIa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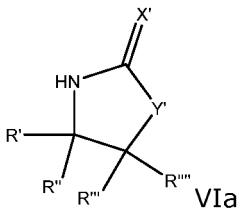
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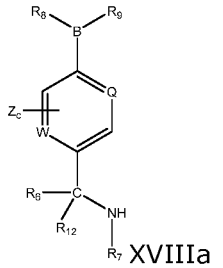
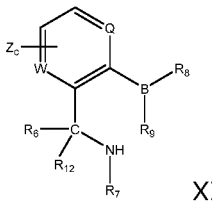

XVIIIa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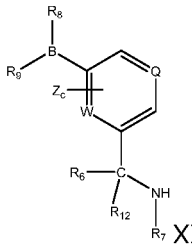
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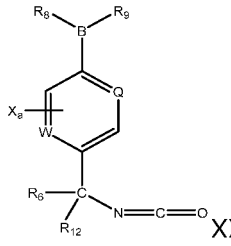
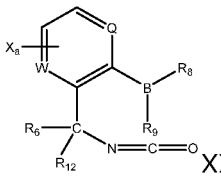
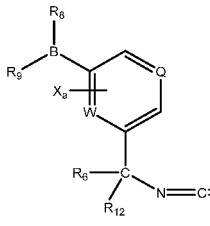

XXIa


XXIIa


XXIIIa

wherein R4, R5, R6, R7, R8, R9, R12, R', R'', R''', R'''', W, Q, X, X', Y, Y' Z, a, b, c are as defined above,

R10 and R11 is a leaving group LG; and

c) Reacting optionally protected MTX (**12**) or optionally protected aminopterin (**31**) with a compound of formula IIa, IIIa, IVa, VIIa, VIIIa, IXa, Xa, XIa, XIIa, XVIIIa, XIXa, XXa, XXIa, XXIIa, or XXIIIa to obtain a compound of formula I according to the invention;
d) Optionally performing a deprotection step;
e) Optionally reacting the compound obtained in step c) or d), as appropriate, with a compound of the formula IIa, IIIa, IVa, VIa, VIIa, VIIIa, IXa, Xa, XIa, XIIa, XVIIIa, XIXa, XXa, XXIa, XXIIa, or XXIIIa, followed by an optional deprotection step to obtain a compound of formula I according to the invention.

Alternatively, providing an optionally protected fragment of MTX (**12**) or aminopterin (**31**) containing the moieties of the formula II, III, IV, V, VI, VII, VIII, IX, X, XI, XII, XIII, XIV, XV, XVI, and/or XVII and reacting it with 6-(bromomethyl)pteridine-2,4-diamine hydrobromide (**33**•HBr), including a final deprotection step when needed, to obtain a compound of the formula I.

Alternatively, optionally reacting methotrexate (**12**), aminopterin (**31**), a protected version of them, or the compound obtained in step c) or d) with a peptide coupling agent e.g. BOP, PyBOP, DCC, EDC, HATU, HOBt, etc. followed by addition of a compound of the formula XVIIIa, XIXa, or XXa, and a final deprotection step when needed, to obtain a compound of the formula I. Suitable coupling agents are known to a person skilled in the art and are disclosed in e.g. *Chem. Rev.*, **2011**, 111 (11), 6557–6602.

In a third aspect the present invention relates to a pharmaceutical composition comprising a compound according to the invention, optionally in combination with one or more excipients.

In a fourth aspect the present invention relates to a compound according to the invention as a prodrug for the treatment of inflammatory diseases or cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows RP-UPLC-MS UV (λ = 306 nm) chromatograms of the activation of prodrug **16**;

FIGURE 2 shows MCF-7 *in vitro* cell viability assay incubated with compounds **12** (methotrexate) and prodrug **16**;

FIGURE 3 shows *in vitro* cell viability study of MCF-7 cells incubated for 48 h with 0.25, 0.062 and 0.015 μM concentrations of compounds **12** (methotrexate) and **16**;

FIGURE 4 shows NCI-H460 *in vitro* cell viability assay incubated with compounds **12** (methotrexate) and prodrug **16**;

FIGURE 5 shows *in vitro* cell viability study of NCI-H460 cells incubated for 48 h with 0.25, 0.062 and 0.015 μM concentrations of compounds **12** (methotrexate) and **16** (mean ± SD, n = 3);

FIGURE 6 shows activation of prodrugs under oxidative conditions (H_2O_2);

FIGURE 7 shows NCI-H460 *in vitro* cell viability assay incubated with compounds **31** (aminopterin) and prodrug **23**;

FIGURE 8 shows MCF-7 *in vitro* cell viability assay incubated with compounds **31** (aminopterin) and prodrug **23**;

FIGURE 9 shows suppression of CIA development in mice after treatment with methotrexate (**MTX, 12**), aminopterin (**AMT, 31**) and prodrugs **16** and **23** (n = 8 per group); and

FIGURE 10 shows the general health of mice was evaluated three times per week during CIA as the average body weight in groups of animals (n = 8) tested with vehicle, **MTX, AMT, 16**, and **23**.

DETAILED DISCLOSURE

Definitions

In the present context the term "alkyl" means a linear, cyclic or branched hydrocarbon group having 1 to 24 carbon atoms, such as methyl, ethyl, propyl, *iso*-propyl, cyclopropyl, butyl, *iso*-butyl, *tert*-butyl, cyclobutyl, pentyl, cyclopentyl, hexyl, and cyclohexyl.

In the present context the term "alkenyl" means a linear, cyclic or branched hydrocarbon groups having 2 to 24 carbon atoms, and comprising (at least) one unsaturated bond. Examples of alkenyl groups are vinyl, allyl, butenyl, pentenyl, hexenyl, heptenyl, octenyl, nonenyl and decaenyl. Preferred examples of alkenyl are vinyl, allyl, butenyl, especially allyl.

In the present context the term “alkynyl” means a linear, cyclic or branched hydrocarbon groups having 2 to 24 carbon atoms, and comprising (at least) one triple bond. Examples of alkynyl groups are acetylene, propynyl, butynyl, pentynyl, and hexynyl.

The term "halogen" includes fluoro, chloro, bromo, and iodo.

In the present context the term “alkoxy” refers to a group –OR, wherein R is alkyl as defined above.

In the present context the term “alkenyloxy” refers to a group –OR, wherein R is alkenyl as defined above.

In the present context the term “aryl” refers to an unsaturated cyclic system. Aryl groups may comprise from 4-12 atoms, suitably from 6-8 atoms, most suitably 6 atoms. “Aryl” is preferably phenyl (-C_6H_5).

In the present context, the term "aromatic" is intended to mean a carbocyclic ring system, such as phenyl, naphthyl, 1,2,3,4-tetrahydronaphthyl, anthracyl, phenanthracyl, pyrenyl, benzopyrenyl, fluorenyl and xanthenyl.

In the present context the term “heteroaryl” refers to an unsaturated cyclic system where one or more of the carbon atoms have been replaced with heteroatoms, e.g. nitrogen (=N- or –NH-), sulphur, and/or oxygen atoms. Heteroaryl groups may comprise from 4-12 atoms, suitably from 5-9 atoms, such as 5-6 atoms, wherein at least one carbon atom has been replaced with a heteroatom, e.g. nitrogen (=N- or –NH-), sulphur, and/or oxygen atoms.

In the present context the term “heteroaromatic” is intended to mean an aromatic carbocyclic ring system where one or more of the carbon atoms have been replaced with heteroatoms, e.g. nitrogen (=N- or –NH-), sulphur, and/or oxygen atoms. Examples of such heteroaryl groups are oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, pyrrolyl, imidazolyl, pyrazolyl, pyridinyl, pyrimidinyl, pyrazinyl, pyridazinyl, triazinyl, coumaryl, furanyl, thienyl, quinolyl, benzothiazolyl, benzotriazolyl, benzodiazolyl, benzooxozolyl, phthalazinyl, phthalanyl, triazolyl, tetrazolyl, isoquinolyl, acridinyl, carbazolyl, dibenzazepinyl, indolyl, benzopyrazolyl, phenoxazonyl. Particularly interesting heteroaryl groups are benzimidazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, pyrrolyl, imidazolyl, pyrazolyl, pyridinyl, pyrimidinyl, pyrazinyl, pyridazinyl, furyl, thienyl, quinolyl, triazolyl, tetrazolyl, isoquinolyl, indolyl in particular benzimidazolyl, pyrrolyl, imidazolyl, pyridinyl, pyrimidinyl, furyl, thienyl, quinolyl, tetrazolyl, and isoquinolyl.

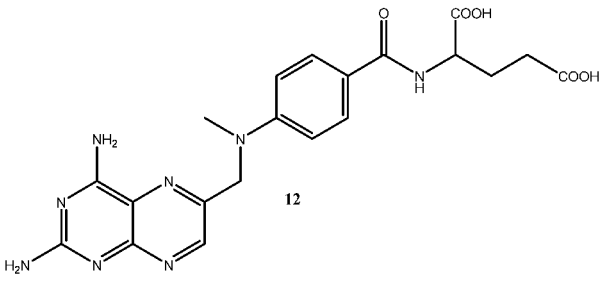
The term "pharmaceutically acceptable salt" is intended to indicate salts prepared by reacting a compound of formula I with a suitable inorganic or organic acid, such as hydrochloric, hydrobromic, hydroiodic, sulfuric, nitric, phosphoric, formic, acetic, 2,2-dichloroacetic, choline, adipic, ascorbic, L-aspartic, L-glutamic, galactaric, lactic, maleic, L-malic, phthalic, citric, propionic, benzoic, glutaric, gluconic, D-glucuronic, methanesulfonic, salicylic, succinic, malonic, tartaric, benzenesulfonic, ethane-1,2-disulfonic, 2-hydroxy ethanesulfonic acid, toluenesulfonic, sulfamic or fumaric acid. Pharmaceutically acceptable salts of compounds of formula I may also be prepared by reaction with a suitable base such as sodium hydroxide, potassium hydroxide, magnesium hydroxide, calcium hydroxide, ammonia, or suitable non-toxic amines, such as lower alkylamines, for example triethylamine, hydroxy-lower alkylamines, for example 2-hydroxyethylamine, bis-(2-hydroxyethyl)-amine, cycloalkylamines, for example dicyclohexylamine, or benzylamines, for example N,N´-dibenzylethylenediamine, and dibenzylamine, or L-arginine or L-lysine.

The term "solvate" is intended to indicate a species formed by interaction between a compound, e.g. a compound of formula I, and a solvent, e.g. alcohol, glycerol or water, wherein said species is in a solid form. When water is the solvent, said species is referred to as a hydrate.

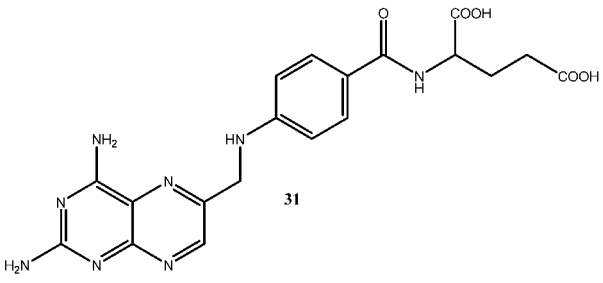
Specific embodiments of the invention

The compounds of the formula I according to the invention may be prepared by the following steps:

a) Providing methotrexate (MTX) of the formula **12** or aminopterin (AMT) of the formula **31** or any protected version of them.;

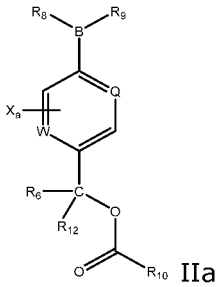


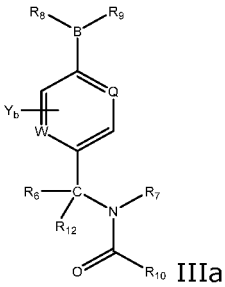
12

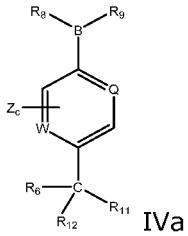


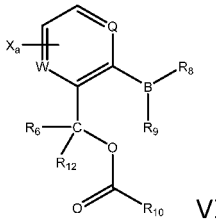
31

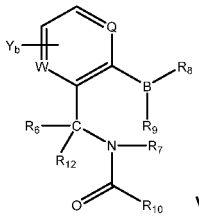
b) Providing a compound of formula IIa, IIIa, IVa, VIa, VIIa, VIIIa, IXa, Xa, XIa, XIIa, XVIIIa, XIXa, XXa, XXIa, XXIIa, or XXIIIa

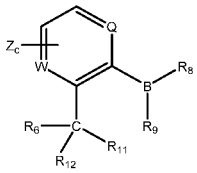

IIa

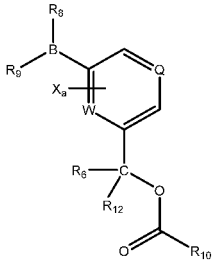

IIIa

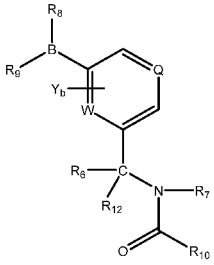

IVa

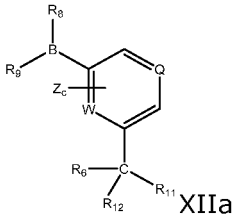

VIIa

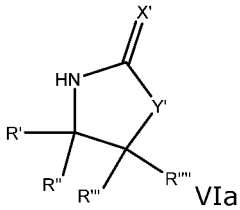

VIIIa


IXa


Xa


XIa


XIIa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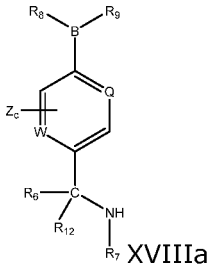
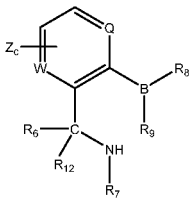
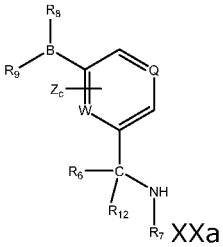
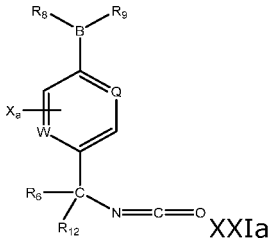
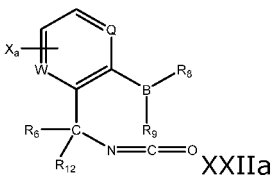
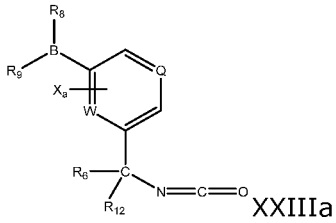

XVIIIa


XIXa


XXa


XXIa


XXIIa


XXIIIa

Wherein R4, R5, R6, R7, R8, R9, R12, R', R'', R''', R'''', W, Q, X, X', Y, Y' Z, a, b, c are as defined above,

R10 and R11 is a leaving group LG; and

c) Reacting optionally protected MTX (**12**) or optionally protected aminopterin (**31**) with a compound of formula IIa, IIIa, IVa, VIIa, VIIIa, IXa, Xa, XIa, XIIa, XVIIIa, XIXa, XXa, XXIa, XXIIa, or XXIIIa to obtain a compound of formula I according to the invention;

d) Optionally performing a deprotection step;

e) Optionally reacting the compound obtained in step c) or d), as appropriate, with a compound of the formula IIa, IIIa, IVa, VIa, VIIa, VIIIa, IXa, Xa, XIa, XIIa, XVIIIa, XIXa, XXa, XXIa, XXIIa, or XXIIIa, followed by an optional deprotection step to obtain a compound of formula I according to the invention.

Alternatively, providing an optionally protected fragment of MTX (**12**) or aminopterin (**31**) containing the moieties of the formula II, III, IV, V, VI, VII, VIII, IX, X, XI, XII, XIII, XIV, XV, XVI, and/or XVII and reacting it with 6-(bromomethyl)pteridine-2,4-diamine hydrobromide (**33**•HBr), including a final deprotection step when needed, to obtain a compound of the formula I.

Step a

Methotrexate (MTX) of the formula **12** or aminopterin of the formula **31** are prepared with protecting groups at the desired positions when necessary. Suitable protective groups are known to a person skilled in the art and are disclosed in e.g. Wuts, P. G. M. & Greene, T. W. Greene's Protective Groups in Organic Synthesis. (Wiley, 2006).

A non-limiting example of the process step a can be found in Preparation Example 8, 9 and 21 below.

Step b

A compound of the formula IIa, IIIa, IVa, VIa, VIIa, VIIIa, IXa, Xa, XIa, XIIa, XVIIIa, XIXa, XXa, XXIa, XXIIa, or XXIIIa may be provided commercially or may be prepared by a method known per se from commercially available starting compounds. Non-limiting examples of possible leaving groups include Cl, Br, I, CDI, p-nitrophenol, etc..

Thus e.g. a compound of the formula IIa, IIIa or IVa may be prepared from the corresponding alcohol, transforming it into reactive species such a chloroformate or halide. Non-limiting examples are shown in Preparation Examples 1, 2 and 3, wherein the synthesis of compounds **1**, **3** and **5** is illustrated.

Thus e.g. a compound of the formula XXIa may be prepared from the corresponding amine, transforming it into the reactive isocyanate. Non-limiting examples are shown in Preparation Example 22, wherein the synthesis of compound **34** is illustrated.

Step c

Optionally protected methotrexate (MTX) of the formula **12** or aminopterin of the formula **31** is reacted with one or more compounds of the formula IIa, IIIa, IVa, VIIa, VIIIa, IXa, Xa, XIa, XIIa, XVIIIa, XIXa, XXa, XXIa, XXIIa, XXIIIa in a manner known per se. Illustrative, non-limiting examples of said reaction can be found in Examples 1, 2, 3, 4, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 below.

Step d

Optionally deprotection of a protected version of methotrexate or aminopterion functionalized with the groups of formula II, III, IV, V, VII, VIII, IX, X, XI, XII, XIII, XIV, XV, XVI and/or XVII, is performed. Illustrative, non-limiting examples of said reaction can be found in Example 6 and 8.

Step e

The compound obtained in step c) or d), as appropriate, may optionally be reacted with a compound of the formula IIa, IIIa, IVa, VIa, VIIa, VIIIa, IXa, Xa, XIa, XIIa, XVIIIa, XIXa, XXa, XXIa, XXIIa, or XXIIIa, followed by an optional deprotection step, to obtain a compound of formula I according to the invention. Illustrative, non-limiting examples of said reaction can be found in Examples 19, 20, 21, 22, 23, 24 below.

Alternatively, providing an optionally protected fragment of MTX (**12**) or aminopterin (**31**) containing the moieties of the formula II, III, IV, V, VI, VII, VIII, IX, X, XI, XII, XIII, XIV, XV, XVI, and/or XVII as illustrated in Preparation Example 14, and reacting it with 6-(bromomethyl)pteridine-2,4-diamine hydrobromide (**33**•HBr) to obtain a compound of the formula I, as illustrated in Example 7, including a final deprotection step when needed, as illustrated in Example 6 and 8.

Alternatively, optionally reacting methotrexate (**12**), aminopterin (**31**), a protected version of them, or the compound obtained in step c) or d) with a peptide coupling agent e.g. BOP, PyBOP, DCC, EDC, HATU, HOBt, etc. followed by addition of a compound of the formula XVIIIa, XIXa, or XXa, and a final deprotection step when needed, to obtain a compound of the formula I. Suitable coupling

agents are known to a person skilled in the art and are disclosed in e.g. *Chem. Rev.*, **2011**, 111 (11), 6557–6602.

An embodiment of the invention is a compound of the formula I, wherein R1 and R2 are independently selected from the group consisting of hydrogen and a moiety of the formula II, III, or IV.

An embodiment of the invention is a compound of the formula I, wherein R3 is selected from the group consisting of hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-4}alkynyl, C_{6-12}aryl, C_{4-11}heteroaryl and a moiety of the formula II, III, or IV.

An embodiment of the invention is a compound of the formula I, wherein R4 and R5 are independently selected from the group consisting of OH, O-C_{1-6}alkyl, O-C_{2-6}alkenyl, O-C_{6-12}aryl, O-C_{4-11}heteroaryl and a moiety of the formula V, or VI

An embodiment of the invention is a compound of the formula I, wherein R1 and R2 are independently selected from the group consisting of hydrogen and a moiety of the formula II, III, or IV;

R3 is selected from the group consisting of hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-4}alkynyl, C_{6-12}aryl, C_{4-11} heteroaryl and a moiety of the formula II, III, or IV;

R4 and R5 are independently selected from the group consisting of OH, O-C_{1-6}alkyl, O-C_{2-6}alkenyl, O-C_{6-12}aryl, O-C_{4-11}heteroaryl and a moiety of the formula V, or VI; and

wherein, if each of R1, R2 and R3 are different from a moiety selected from a moiety of the formula II, III, and IV, then at least one of R4 and R5 is a moiety of the formula V.

An embodiment of the invention is a compound of the formula I, wherein W and Q are both C.

Another embodiment of the invention is a compound of the formula I, wherein W is C and Q is N.

Another embodiment of the invention is a compound of the formula I, wherein W is N and Q is C.

Another embodiment of the invention is a compound of the formula I, wherein W and Q are both N.

An embodiment of the invention is a compound of formula I, wherein R6 and R7 are independently selected from the group consisting of hydrogen and C_{1-6}alkyl, preferably selected from the group consisting of hydrogen and C_{1-4}alkyl, preferably selected from the group consisting of hydrogen and methyl, preferably wherein R6 and R7 are both hydrogen.

An embodiment of the invention is a compound of formula I, wherein R8 and R9 are independently hydroxyl groups or R8 and R9 form, together with the intervening B and O atoms, a pinacol or catechol group, preferably wherein R8 and R9 are independently hydroxyl groups or R8 and R9 form, together with the intervening B and O atoms, a pinacol group, preferably wherein R8 and R9 are both hydroxyl groups.

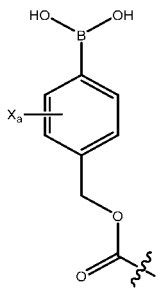
An embodiment of the invention is a compound of formula I, wherein each of X, Y and Z are selected from the group consisting of halogen, cyano, hydroxyl, CF_3, and C_{1-6}alkyl; and each of a, b and c are 0, 1 or 2.

An embodiment of the invention is a compound of formula I, wherein each of X, Y and Z are selected from the group consisting of halogen, cyano, hydroxyl, and C_{1-6}alkyl; and each of a, b and c are 0 or 1.

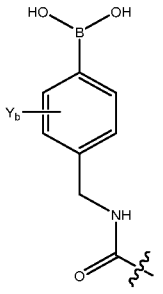
An embodiment of the invention is a compound of formula I, wherein each of X, Y and Z are selected from the group consisting of halogen and C_{1-4}alkyl; and each of a, b and c are 0 or 1.

An embodiment of the invention is a compound of formula I, wherein each of X, Y and Z are selected from the group consisting of fluoro and methyl; and each of a, b and c are 0 or 1.

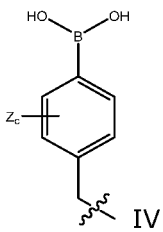
An embodiment of the invention is a compound of the formula I, wherein R2 is selected from the group consisting of



II



III and



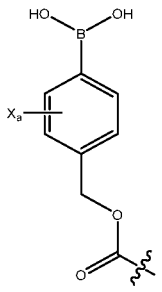
IV

in which X, Y, Z, a, b and c, are as defined above;

R1 is hydrogen;

R3 is selected from the group consisting of hydrogen and C_{1-6}alkyl, preferably selected from the group consisting of hydrogen and C_{1-4}alkyl, preferably wherein said R3 is methyl; and
R4 and R5 are selected from the group consisting of OH and O-C_{1-6}alkyl, preferably selected from the group consisting of OH and O-C_{1-4}alkyl, preferably wherein R4 and R5 are both methoxy or hydroxy.

An embodiment of the invention is a compound of the formula I, wherein R3 is selected from the group consisting of



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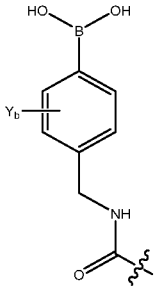
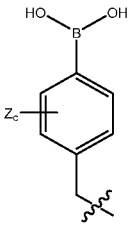


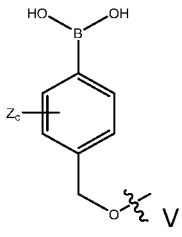
III and



IV

in which X, Y, Z, a, b, and c are as defined above;
R1 and R2 are hydrogen; and
R4 and R5 are selected from the group consisting of OH and O-C_{1-6}alkyl, preferably selected from the group consisting of OH and O-C_{1-4}alkyl, preferably wherein R4 and R5 are both methoxy or hydroxy.

An embodiment of the invention is a compound of the formula I, wherein R4 and/or R5 is a moiety of the formula



V

in which Z and c are as defined above;
R1 and R2 are hydrogen; and
R3 is selected from the group consisting of hydrogen and C_{1-6}alkyl, preferably selected from the group consisting of hydrogen and C_{1-4}alkyl, preferably wherein said R3 is methyl.

An embodiment of the invention is a compound of the formula I, wherein Y' is S.

An embodiment of the invention is a compound of the formula I, wherein X' is O.

An embodiment of the invention is a compound of the formula I, wherein R' and R'' are both hydrogen.

An embodiment of the invention is a compound of the formula I selected from the group consisting of

(*S*)-(4-((((2-Amino-6-(((4-((1,5-dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)carbamoyl)oxy)methyl) phenyl)boronic acid (compound **9**)

(*S*)-(4-((((2-Amino-6-(((4-((1,5-dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)carbamoyl)oxy)methyl)-2-methylphenyl)boronic acid (compound **10**)

(*S*)-(4-((((2-Amino-6-(((4-((1,5-dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)carbamoyl)oxy)methyl)-2-fluorophenyl)boronic acid (compound **11**)

Bis(4-methoxybenzyl) (4-(((2-amino-4-((((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)carbonyl)amino)pteridin-6-yl)methyl)(methyl)amino)benzoyl)-*L*-glutamate (compound **14**)

(*S*)-(4-((((2-Amino-6-(((4-((1,5-bis((4-methoxybenzyl)oxy)-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)carbamoyl)oxy)methyl)phenyl)boronic acid (compound **15**)

(4-(((2-Amino-4-((((4-boronobenzyl)oxy)carbonyl)amino)pteridin-6-yl)methyl)(methyl)amino)benzoyl)-*L*-glutamic acid (compound **16**)

(S)-(4-((((2,4-Diaminopteridin-6-yl)methyl)(4-((1,5-dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)amino)methyl)phenyl)boronic acid (compound **22**)

(4-((4-Boronobenzyl)((2,4-diaminopteridin-6-yl)methyl)amino)benzoyl)-*L*-glutamic acid (compound **23**)

(*S*)-(4-((((2-Amino-6-(((4-((1,5-di-*tert*-butoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)carbamoyl)oxy)methyl)phenyl)boronic acid (compound **30**)

(4-((((2-Amino-6-(((4-((1,5-bis(allyloxy)-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)carbamoyl)oxy)methyl)phenyl)boronic acid (compound **33**)

(*S*)-(4-((3-(2-Amino-6-(((4-((1,5-dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)ureido)methyl)phenyl)boronic acid **(**compound **35)**

(S)-5-((4-Boronobenzyl)oxy)-4-(4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)benzamido)-5-oxopentanoic acid **(**compound **37)**

(S)-5-((4-Boronobenzyl)oxy)-2-(4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)benzamido)-5-oxopentanoic acid **(**compound **38)**

(*S*)-(4-(((5-((4-Boronobenzyl)oxy)-2-(4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)benzamido)-5-oxopentanoyl)oxy)methyl)phenyl)boronic acid **(**compound **39)**

(*S*)-(4-(((2-Amino-6-(((4-((1,5-dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)amino)methyl)phenyl)boronic acid **(40)**

(*S*)-(4-(((4-Amino-6-(((4-((1,5-dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)-phenyl)(methyl)amino)methyl)pteridin-2-yl)amino)methyl)phenyl)boronic acid **(**compound **41)**

(*S*)-((((6-(((4-((1,5-Dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)-(methyl)amino)methyl)pteridine-2,4-diyl)bis(azanediyl))bis(methylene))bis(4,1-phenylene))diboronic acid **(**compound **42)**

(*S*)-((((((6-(((4-((1,5-Dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)-(methyl)amino)methyl)pteridine-2,4-diyl)bis(azanediyl))bis(carbonyl))bis(oxy))-bis(methylene))bis(4,1-phenylene))diboronic acid **(**compound **43)**

(2-(4-(((2-Amino-4-((((4-boronobenzyl)oxy)carbonyl)amino)pteridin-6-yl)methyl)(methyl)amino)benzamido)-5-oxo-5-(2-oxothiazolidin-3-yl)pentanoic acid **(**compound **44)**

4-(4-(((2-Amino-4-((((4-boronobenzyl)oxy)carbonyl)amino)pteridin-6-yl)methyl)(methyl)amino)benzamido)-5-oxo-5-(2-oxothiazolidin-3-yl)pentanoic acid **(**compound **45)**

(4-((((2-Amino-6-(((4-((1,5-dioxo-1,5-bis(2-oxothiazolidin-3-yl)pentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)carbamoyl)oxy)-methyl)phenyl)boronic acid **(**compound **46)**

2-(4-((4-Boronobenzyl)((2,4-diaminopteridin-6-yl)methyl)amino)benzamido)-5-oxo-5-(2-oxothiazolidin-3-yl)pentanoic acid **(**compound **47)**

4-(4-((4-Boronobenzyl)((2,4-diaminopteridin-6-yl)methyl)amino)benzamido)-5-oxo-5-(2-oxothiazolidin-3-yl)pentanoic acid **(**compound **48)**

(4-((((2,4-Diaminopteridin-6-yl)methyl)(4-((1,5-dioxo-1,5-bis(2-oxothiazolidin-3-yl)pentan-2-yl)carbamoyl)phenyl)amino)methyl)phenyl)boronic acid **(**compound **49)**.

Pharmaceutically acceptable salts, solvates, and stereoisomers of the compound of formula I are also provided.

Compounds of formula I may comprise asymmetrically substituted (chiral) carbon atoms and carbon-carbon double bonds which may give rise to the existence of stereoisomeric forms, e.g. enantiomers, diastereomers and geometric isomers. The present invention includes all such isomers, either in pure form or as mixtures thereof.

The compounds of formula I may be obtained in crystalline form either directly by concentration from an organic solvent or by crystallisation or recrystallisation from an organic solvent or mixture of said solvent and a cosolvent that may be organic or inorganic, such as water. The crystals may be isolated in essentially solvent-free form or as a solvate, such as a hydrate. The invention covers all crystalline modifications and forms and also mixtures thereof.

The present invention further relates to a pharmaceutical composition comprising as an active ingredient an effective amount of at least one compound of the Formula I, or pharmaceutically acceptable salt thereof and/or stereoisomer thereof, optionally in combination with one or more

conventional excipients. The pharmaceutical composition of the present invention usually comprises 0.1-90wt% of the compound of Formula I and/or physiologically acceptable salt thereof. The pharmaceutical composition can be prepared according to methods known in the art. For this purpose, if necessary, the compound of Formula I and/or a stereoisomer thereof is combined with one or more solid or liquid pharmaceutically acceptable excipients and/or adjuvants, to form an application form or dosage form suitable for administration to a human.

The compound of Formula I of the present invention or the pharmaceutical composition containing the same can be administered in unit dosage form, and the administration routes can be intestinal or parenteral administration, such as oral, intramuscular, subcutaneous, nasal, oral mucosal, skin, intraperitoneal or rectal administration. The administration dosage form can be, for example, tablets, capsules, drop pills, aerosols, pills, powders, solutions, suspensions, emulsions, granules, liposomes, transdermal agents, buccal tablets, suppositories, lyophilized powder injections, can be normal preparations, sustained-release preparations, controlled-release preparations, and various microparticle administration systems. In order to process the unit dosage form into tablets, various carriers well known in the art can be widely used. The examples of the carriers can be, for example, diluents and absorbents, such as starch, dextrin, calcium sulfate, lactose, mannitol, sucrose, sodium chloride, glucose, urea, calcium carbonate, kaolin, microcrystalline cellulose, aluminum silicate; wetting agent and binding agent, such as water, glycerol, polyethylene glycol, ethanol, propanol, starch slurry, dextrin, syrup, honey, glucose solution, acacia mucilage, gelatin mucilage, sodium carboxymethylcellulose, shellac, methylcellulose, potassium phosphate, polyvinylpyrrolidone; disintegrants, such as, dry starch powder, alginate, agar powder, laminarin powder, sodium hydrogen carbonate and citric acid, calcium carbonate, polyoxyethylene sorbitol fatty acid ester, sodium dodecyl sulfate, methyl cellulose, ethyl cellulose; disintegration inhibitors, such as sucrose, tristearin, cocoa butter, hydrogenated oil; absorption enhancers, such as, quaternary ammonium salts, sodium dodecyl sulfate; lubricants, such as, talc, silica, maize powder, stearate, boric acid, liquid paraffin, polyethylene glycol. The tablets can be further processed into coated tablets, for example, sugar coated tablets, thin film coated tablets, enteric-coated tablets, or double-layer tablets or multi-layer tablets. In order to process the administration unit into pills, various carriers known in the art can be used. The examples of the carriers can be, for example, diluents and absorbing agents, such as glucose, lactose, starch, cocoa butter, hydrogenated vegetable oil, polyvinylpyrrolidone, Gelucire, kaolin, talc; binding agent, such as acacia gum, tragacanth gum, gelatin, ethanol, honey, liquid sugar, rice paste or panada; disintegrants, such as agar powder, dry starch powder, alginate, sodium dodecyl sulfonate, methyl cellulose, ethyl cellulose. In order to process the administration unit into suppositories, various carriers known in the art can be widely used. The examples of the carriers can be, for example, polyethylene glycol, lecithin, cocoa butter, fatty alcohol, ester of fatty alcohol, gelatin, semi-synthetic ester. In order to process the administration unit into capsules, the compound of Formula I or stereoisomer thereof as effective

component is mixed with the various carriers, and the resultant mixture is placed in hard gelatin capsule shells or soft capsules. The compound of Formula I or stereoisomer thereof as effective component can also be processed into microcapsules, suspended in aqueous medium to form a suspension, or placed in hard capsules or processed into injections. In order to process the administration unit into a preparation for injection, such as solution, emulsion, lyophilized powder injection and suspension, all diluents known in the art, for example, water, ethanol, polyethylene glycol, 1,3-propylene glycol, ethoxylated isostearyl alcohol, multi-oxidized isostearyl alcohol, polyoxyethylene sorbitol fatty acid ester, could be used. In addition, in order to prepare an isotonic injection solution, an suitable amount of sodium chloride, glucose or glycerol can be added to the injection preparation, and conventional co-solvent, buffer agent, and pH regulator can further added.

In addition, if necessary, colouring agents, preservatives, flavoring agents, correctants, sweetening agents or other materials can also be added to the pharmaceutical compositions.

The administration dose of the compound of Formula I, or a stereoisomer thereof may depend on many factors, for example, the properties and severity of the diseases to be prevented or treated, the gender, age, bodyweight and individual reaction of patient or animal, the specific compound to be used, the administration routes and times, and so on. The dose can be of single dose form or can be divided into several dose forms, such as, two, three or four dose forms.

The compounds according to the invention may be used as a prodrug for the treatment of inflammatory diseases or cancer. Non-limiting examples of inflammatory diseases include rheumatoid arthritis (RA), juvenile dermatomyositis, juvenile rheumatoid arthritis, psoriasis, psoriatic arthritis, lupus, sarcoidosis, atopic dermatitis, eczema, Crohn's disease, uveitis associated with juvenile idiopathic arthritis or ulcerative colitis, colitis ulcerosa, multiple sclerosis, Amyotropic Lateral Sclerosis (ALS), non-infectious ocular inflammation, vasculitis, systemic lupus erythematosus, and eosinophilic fasciitis. Non-limiting examples of cancer diseases include acute lymphocytic leukemia, meningeal leukemia, myeloproliferative neoplasm, breast cancer, squamous cell carcinoma, lymphosarcoma, osteosarcoma, advanced mycosis fungoides (cutaneous T cell lymphoma), small cell types lung cancer, non-small cell lung cancer, and non-Hodgkin's lymphoma.

General Methods

Unless otherwise stated, commercially available reagents were used without further purification and all solvents were of HPLC quality. Reactions under nitrogen atmosphere were performed in oven- or flame-dried glassware and dry solvents. Anhydrous CH_2Cl_2, CH_3CN, THF, DMF, and toluene were obtained from Innovative Technology PS-MD-7 Pure-solve solvent purification system. Dry solvents

were typically prepared by drying over molecular sieves (3 Å or 4 Å). All reactions were monitored by thin-layer chromatography (TLC) and reversed-phased ultra-performance liquid chromatography mass spectrometry (RP-UPLC-MS).

Analytical TLC was conducted on Merck aluminium sheets covered with silica (C60). The plates were either visualized under UV-light or stained by dipping in a developing agent followed by heating. $KMnO_4$ (3 g in water (300 mL) along with K_2CO_3 (20 g) and 5% aqueous NaOH (5 mL)) or Ninhydrin (3 g in a mixture of *n*-butanol (200 mL) and AcOH (6 mL)) were used as developing agents. Flash column chromatography was performed using Matrex 60 Å, 35-70 µ silicagel.

All new compounds were characterized by ^1H NMR, ^{13}C NMR and HRMS (ESI). For recording of ^1H NMR and ^{13}C NMR a Bruker Ascend with a Prodigy cryoprobe (operating at 400 MHz for proton and 100 MHz for carbon) was used. The chemical shifts (δ) are reported in parts per million (ppm) and the coupling constants (*J*) in Hz. For spectra recorded in $CDCl_3$, signal positions were measured relative to the signal for $CHCl_3$ (7.26 ppm for ^1H NMR and 77.16 ppm for ^{13}C NMR). For spectra recorded in DMSO-d_6 signal positions were measured relative to the signal for DMSO (δ 2.50 ppm for ^1H NMR and 39.52 ppm for ^{13}C NMR). For spectra recorded in C_6D_6, signal positions were measured relative to the signal for C_6H_6 (7.16 ppm for ^1H NMR and 128.06 ppm for ^{13}C NMR) For spectra recorded in D_2O signal positions were measured relative to the signal for H_2O (δ 4.79 ppm for ^1H NMR). Analytical RP-UPLC-MS (ESI) analysis was performed on a Waters AQUITY RP-UPLC system equipped with a diode array detector using a Thermo accucore C18 column (d 2.6 µm, 2.1 x 50 mm; column temp: 50 °C; flow: 0.6 mL/min).

Four different methods were use. Method A: eluents A (0.1% HCO_2H in mili-Q water) and B (0.1% HCO_2H in CH_3CN) were used in a linear gradient (5% B to 100% B) in a total run time of 2.6 min. Method B: eluents A (0.1% HCO_2H in H_2O) and B (0.1% HCO_2H in CH_3CN) were used in a linear gradient (5% B to 100% B) in a total run time of 5.0 min. Method C: eluents A (10mM NH_4OAc in mili-Q water) and B (0.1% NH_4OAc in mili-Q water /MeCN, 90/10, v/v) were used in a linear gradient (5% B to 100% B) in a total run time of 2.6 min. Method D: eluents A (0.1% NH_4OAc in H_2O) and B (0.1% NH_4OAc in CH_3CN) were used in a linear gradient (5% B to 100% B) in a total run time of 5.0 min

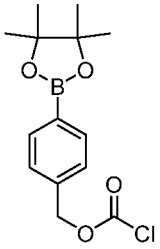
. The LC system was coupled to a SQD mass spectrometer operating in both positive and negative electrospray modes. The temperature for all recordings was approximately 20 °C. Analytical LC-HRMS (ESI) analysis was performed on an Agilent 1100 RP-LC system equipped with a diode array detector using a Phenomenex Luna C18 column (*d* 3 µm, 2.1 x 50 mm; column temp: 40 °C; flow: 0.4 mL/min). Eluents A (0.1% HCO_2H in H_2O) and B (0.1% HCO_2H in CH_3CN) were used in a linear gradient (20% B to 100% B) in a total run time of 15 min. The LC system was coupled to a

Micromass LCT orthogonal time-of-flight mass spectrometer equipped with a Lock Mass probe operating in positive or negative electrospray mode.

Purification of reactions by preparative RP-HPLC was performed on a Waters Alliance reverse-phase HPLC system consisting of a Waters 2545 Binary Gradient Module equipped with either an xBridge BEH C18 OBD Prep Column (130 Å, 5 µm, 30 x 150 mm) or an xBridge Peptide BEH C18 OBD Prep Column (130 Å, 5 µm, 19 mm x 100 mm) both operating at 20 °C and a flow rate of 20 mL/min, a Waters Photodiode Array Detector (detecting at 210-600 nm), a Waters UV Fraction Manager, and a Waters 2767 Sample Manager. Elution was carried out in a reversed-phase gradient fashion combining A1 (0.1% HCO_2H in mili-Q water) and B1 (0.1% HCO_2H in CH_3CN) or A2 (5 mM NH_4OAc in H_2O) and B2 (5 mM NH_4OAc in CH_3CN): 5% B to 70 % B in 10 min, hold for 3.5 min, then 70% B to 100% B in 1.5 min, and hold 3 minutes. Total run time: 20 min.

PREPARATION EXAMPLE 1

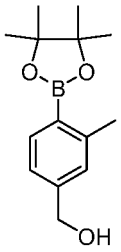
4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl carbonochloridate (1)



A solution of (4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)methanol (2.0 g, 8.54 mmol) in anhydrous dioxane (25 mL) was treated with phosgene (20% in toluene, 22.6 mL, 42.7 mmol) and the reaction was stirred under a N_2 atmosphere at 21 °C for 20 h. The mixture was concentrated *in vacuo*, redissolved, and co-evaporated with toluene (2x) to afford the crude product **1** (2.5 g, quantitative yield) as a clear oil. The crude chloroformate was used in subsequent reactions within few hours after its isolation. ^1H NMR (400 MHz, $CDCl_3$) δ 7.84 (d, *J* = 8.1 Hz, 2H), 7.38 (d, *J* = 8.1 Hz, 2H), 5.31 (s, 2H), 1.35 (s, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.8, 136.2, 135.4, 128.0, 84.2, 73.4, 25.0.

PREPARATION EXAMPLE 2

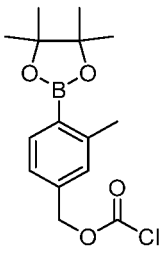
(3-Methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)methanol (2)



A suspension of (4-(hydroxymethyl)-2-methylphenyl)boronic acid (0.9 g, 5.42 mmol) and pinacol (0.7 g, 5.96 mmol) in anhydrous THF (25 mL) was refluxed for 16 h under a N_2 atmosphere. The solids dissolved during the reaction. The mixture was concentrated *in vacuo* and the residue was purified by flash column chromatography on silica gel using a mixture of CH_2Cl_2/EtOAc (9:1, v/v) as the eluent to give the title compound **2** as a clear oil (1.27 g, 95%). R_f = 0.84 (silica, eluent CH_2Cl_2/EtOAc, 1/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.4 Hz, 1H), 7.17 – 7.09 (m, 2H), 4.67 (s, 2H), 2.54 (s, 3H), 1.34 (s, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.5, 143.6, 136.4, 128.3, 123.2, 83.6, 65.4, 25.0, 22.3; HRMS (ESI) *m/z*: calcd for $C_{14}H_{22}BO_3$ $[M+Na]^+$ 271.1476, found 271.1512.

PREPARATION EXAMPLE 3

3-Methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl carbonochloridate (3)

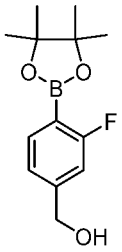


A solution of (3-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)methanol (**2**) (1.2 g, 4.84 mmol) in anhydrous dioxane (25 mL) was treated with phosgene (20% in toluene,

12.8 mL, 24.2 mmol) and the reaction was stirred at 21 °C under a N_2 atmosphere for 6 h. The mixture was then concentrated *in vacuo*, redissolved and co-evaporated with toluene (2x) to afford the crude product **3** as a clear oil (1.5 g, quantitative yield). The crude chloroformate was used in subsequent reactions within few hours after its isolation. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, *J* = 8.0 Hz, 1H), 7.20 – 7.13 (m, 2H), 5.26 (s, 2H), 2.55 (s, 3H), 1.34 (s, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.8, 145.7, 136.5, 135.7, 130.1, 125.0, 83.8, 73.5, 25.0, 22.3.

PREPARATION EXAMPLE 4

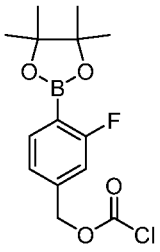
(3-Fluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)methanol (4)



A suspension of (2-fluoro-4-(hydroxymethyl)phenyl)boronic acid (0.90 g, 5.30 mmol) and pinacol (0.69 g, 5.83 mmol) in anhydrous THF (25mL) was refluxed for 16 h under a N_2 atmosphere. The solids dissolved during this time and then the solvent was removed *in vacuo*. The residue was purified by flash column chromatography on silica gel using a mixture of CH_2Cl_2/EtOAc (9/1, v/v) as the eluent to give the title compound **4** as a clear oil (1.26 g, 94%). The oil became a white solid upon storage in the fridge. R_f = 0.71 (silica, eluent CH_2Cl_2/EtOAc, 1/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 – 7.67 (m, 1H), 7.12 (d, *J* = 7.6 Hz, 1H), 7.06 (d, *J* = 10.1 Hz, 1H), 4.72 (s, 2H), 1.36 (s, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.6 (d, *J* = 251.4 Hz), 147.2 (d, *J* = 8.0 Hz), 137.2 (d, *J* = 8.4 Hz), 121.7 (d, *J* = 2.9 Hz), 113.4 (d, *J* = 24.7 Hz), 84.0, 64.6, 25.0.

PREPARATION EXAMPLE 5

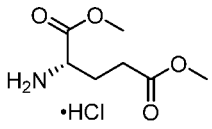
4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2 yl)benzyl carbonochloridate (5)



To a solution of (3-fluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)methanol (**4**) (1.2 g, 4.8 mmol) in anhydrous dioxane (25 mL) was added phosgene (15% in toluene, 17 mL) and the mixture was stirred under a N_2 atmosphere for 6 h at 21 °C. The mixture was then concentrated *in vacuo* and the residue co-evaporated with toluene (2x) to afford the crude product **5** as a white solid (1.5 g, quantitative yield). The crude chloroformate was used in subsequent reactions within few hours after isolation. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (dd, *J* = 7.5, 6.1 Hz, 1H), 7.15 (dd, *J* = 7.5, 1.3 Hz, 1H), 7.07 (dd, *J* = 9.6, 1.1 Hz, 1H), 5.29 (s, 2H), 1.36 (s, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.7 (d, *J* = 251 Hz), 150.8, 138.92 (d, *J* = 8.3 Hz), 137.6 (d, *J* = 8.5 Hz), 123.5 (d, *J* = 3.2 Hz), 115.3 (d, *J* = 25.4 Hz), 84.3 (s), 72.2 (s), 25.0 (s).

PREPARATION EXAMPLE 6

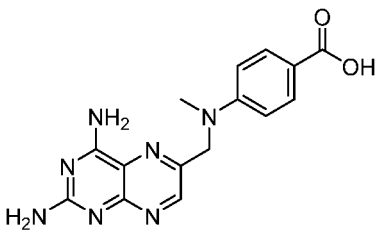
Dimethyl L-glutamate hydrochloride (6·HCl)



Thionyl chloride (5.0 mL, 68 mmol) was added dropwise to 50 mL of anhydrous MeOH at 0 °C. The mixture was stirred for 30 min at the same temperature followed by addition of L-glutamic acid **17** (5.0 g, 34 mmol). The reaction was stirred under a N_2 atmosphere for 3 days at 21 °C and concentrated *in vacuo* to afford **6**·HCl (7.19 g, quant.) as a colorless solid. The crude product was used without further purification. ^{1}H NMR (400 MHz, D_2O) δ 4.24 – 4.19 (m, 1H), 3.85 (s, 3H), 3.73 (s, 3H), 2.65 (td, *J* = 7.3, 2.2 Hz, 2H), 2.37 – 2.13 (m, 2H); ^{13}C NMR (101 MHz, D_2O) δ 174.8, 170.2, 53.6, 52.4, 52.0, 29.2, 24.7.

PREPARATION EXAMPLE 7

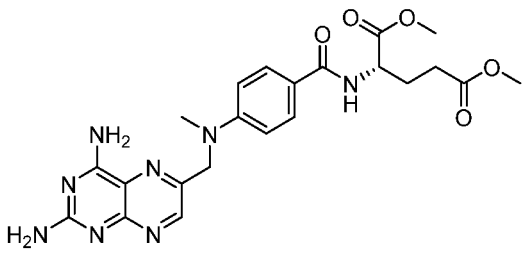
4-(((2,4-Diaminopteridin-6-yl)methyl)(methyl)amino)benzoic acid (7)



2,4-diamino-6-(hydroxymethyl)pteridine hydrochloride (4.40 g, 19.2 mmol) was dissolved in hot water (150 mL) and after cooling to 21 °C the solution was neutralized with 1M NaOH aq. solution to pH 7 (ca. 20 mL). The formed precipitates were collected by filtration, washed with water, and dried *in vacuo* over P_2O_5 to afford an orange-beige solid corresponding to 2,4-diamino-6-(hydroxymethyl)pteridine. The solid was suspended in dry DMAc (25 mL) and triphenylphosphine dibromide (18.1 g, 42.9 mmol) was added to the suspension. The turbid and dark mixture was stirred for 24 h at 20 °C under a N_2 atmosphere. Then 4-aminobenzoic acid (2.97 g, 19.6 mmol) was added to the reaction and stirred for 3 additional days. The reaction mixture was poured into 250 mL of 0.33M NaOH and the precipitate was filtered off. The filtrate was neutralized with 10% aq. acetic acid (ca. 20 mL and the precipitate form upon neutralization was filtered, washed with water, triturated with MeOH, filtered, and dried *in vacuo* to afford **7** as an orange-beige solid (5.70 g, 91%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.15 (br s, 1H), 8.63 (s, 1H), 8.17 (br s, 1H), 7.94 (br s, 1H), 7.73 (d, *J* = 9.0 Hz, 2H), 7.04 (br s, 2H), 6.83 (d, *J* = 9.0 Hz, 2H), 4.81 (s, 2H), 3.23 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.8, 163.2, 161.1, 152.3, 149.5, 147.8, 131.5, 122.2, 118.1, 111.7, 100.0, 55.2, 39.6.

PREPARATION EXAMPLE 8

Dimethyl (4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)benzoyl)-L-glutamate (8)

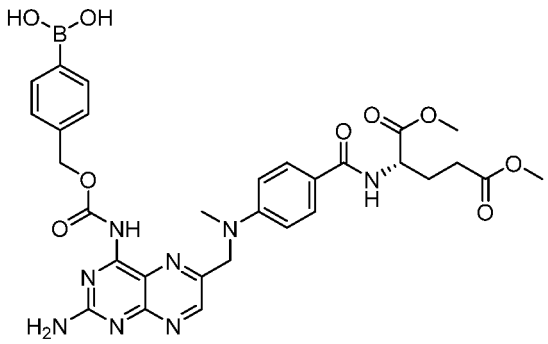


To a solution of 4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)benzoic acid (**7**) (3.0 g, 9.2 mmol) in anhydrous DMF (80 mL) was added Et_3N (6.4 mL, 46.1 mmol) followed by PyBOP (6.48 g, 12.4 mmol). The mixture was stirred at 21 °C under a N_2 atmosphere for 30 min. Dimethyl L-glutamate hydrochloride (**6**·HCl) (2.1 g, 9.9 mmol) was added and the reaction mixture was stirred for 5 h. The crude mixture was filtered through a path of celite to remove solids and the filtrate concentrated *in vacuo*. The solid residue was triturated with a mixture of EtOAc/$CHCl_3$ (150 mL, 1/1) and poured into 750 mL of Et_2O at 0 °C under strong stirring. The resultant suspension was filtered, washed with Et_2O and cold water, triturated with hot MeOH, and filtered again. The resultant orange solid wash purified by flash column chromatography on silica gel using a mixture of CH_2Cl_2/MeOH (92.5/7.5, v/v) as the eluent to give the title compound **8** (2.39 g, 54%) as a yellow

solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 (s, 1H), 8.34 (d, *J* = 7.4 Hz, 1H), 7.72 (d, *J* = 8.8 Hz, 2H), 7.65 (br s, 1H), 7.43 (br s, 1H), 6.82 (d, *J* = 8.8 Hz, 2H), 6.61 (br s, 2H), 4.78 (s, 2H), 4.39 (ddd, *J* = 9.4, 7.4, 5.6 Hz, 1H), 3.61 (s, 3H), 3.57 (s, 3H), 3.21 (s, 3H), 2.41 (t, *J* = 7.4 Hz, 2H), 2.17 – 2.03 (m, 1H), 2.01 – 1.72 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.7, 172.6, 166.4, 162.9, 162.7, 155.2, 151.0, 149.2, 145.9, 129.0, 121.4, 120.7, 111.0, 54.9, 51.8, 51.7, 51.4, 38.7, 30.0, 25.8; HRMS (ESI) *m/z*: calcd for $C_{22}H_{27}N_8O_5$ $[M+H]^+$ 483.2099, found 483.2119.

EXAMPLE 1

(S)-(4-((((2-Amino-6-(((4-((1,5-dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)carbamoyl)oxy)methyl) phenyl)boronic acid (9)

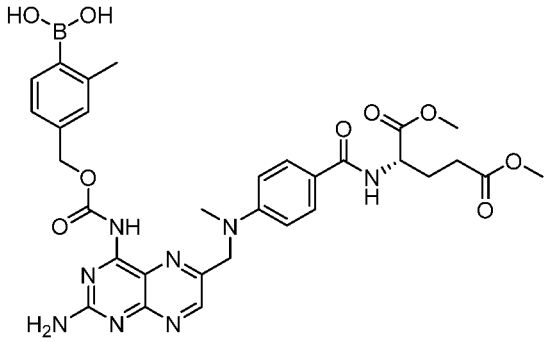


To a suspension of dimethyl (4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)benzoyl)-*L*-glutamate hydrochloride (**8**) (313 mg, 0.68 mmol) in dry CH_2Cl_2 (25 mL) was added DMAP (417 mg, 3.42 mmol) followed by DIPEA (0.60 mL, 3.42 mmol). The mixture was cooled to 0 °C and a solution of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl carbonochloridate (**1**) (1.01 g, 3.42 mmol) in CH_2Cl_2 (10 mL) was added dropwise. The reaction was allowed to warm to 21 °C and stirred for 5 h under a N_2 atmosphere. The mixture was diluted with CH_2Cl_2 (100 mL), washed with 1M HCl (2 x75 mL), sat. $NaHCO_3$ (2 x 75mL), and brine (75 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford a yellow solid (770 mg) that was purified by preparative HPLC. The CH_3CN/H_2O fractions containing the pinacolate intermediate were poured together into a 250mL round bottom flask and HCl cc. (0.3 mL, ca. pH 2) was added. The reaction mixture was stirred for 16 h at 21 °C and quenched with sat. $NaHCO_3$ (ca. 50 mL). After removal of the CH_3CN *in vacuo* the formed precipitate was filtered, washed with H_2O and dried i*n vacuo* to afford the title compound **9** (122 mg, 27%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.88 (br s, 1H), 8.68 (s, 1H), 8.34 (d, *J* = 7.5 Hz, 1H), 8.08 (s, 2H), 7.82 (d, *J* = 7.8 Hz, 2H), 7.72 (d, *J* = 8.8 Hz, 2H), 7.43 (d, *J* = 7.8 Hz, 2H), 7.27 (br s, 2H), 6.82

(d, *J* = 8.9 Hz, 2H), 5.24 (s, 2H), 4.84 (s, 2H), 4.39 (ddd, *J* = 9.3, 7.7, 5.7 Hz, 1H), 3.60 (s, 3H), 3.56 (s, 3H), 3.20 (s, 3H), 2.40 (t, *J* = 7.4 Hz, 2H), 2.15 – 1.90 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.7, 172.6, 166.4, 162.0, 157.8, 155.1, 151.1, 150.7, 150.3, 148.0, 137.7, 134.2, 129.0, 127.0, 120.9, 120.9, 111.1, 66.7, 54.9, 51.8, 51.7, 51.4, 38.9, 30.0, 25.8. HRMS (ESI) *m/z*: calcd for $C_{30}H_{34}BN_8O_9$ $[M+H]^+$ 661.2536, found 661.2566.

EXAMPLE 2

(S)-(4-((((2-Amino-6-(((4-((1,5-dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)carbamoyl)oxy)methyl)-2-methylphenyl)boronic acid (10)

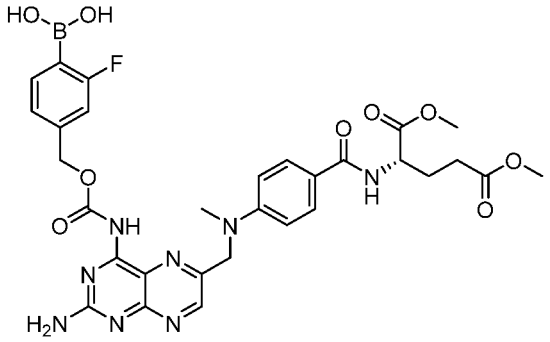


To a suspension of dimethyl (4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)benzoyl)-*L*-glutamate hydrochloride (**8**) (427 mg, 0.93 mmol) in dry CH_2Cl_2 (25 mL) was added DMAP (569 mg, 4.66 mmol) followed by DIPEA (0.81 mL, 4.66 mmol). The mixture was cooled to 0 °C and a solution of 3-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl carbonochloridate (**3**) (1.45 g, 4.66 mmol) in CH_2Cl_2 (10 mL) was added dropwise. The reaction was allowed to warm to 21 °C and stirred for 5 h under a N_2 atmosphere. The mixture was diluted with CH_2Cl_2 (100 mL), washed with 1M HCl (2 x75 mL), sat. $NaHCO_3$ (2 x 75mL) and brine (75 mL), dried over Na_2SO_4, filtered and dried over *in vacuo*. The solid residue was purified by preparative HPLC. The CH_3CN/H_2O fractions containing the pinacolate intermediate were poured together into a 250mL round bottom flask and HCl cc. (0.3 mL, ca. pH 2) was added. The reaction mixture was stirred for 16h at room temperature and quenched with sat. $NaHCO_3$ (ca. 50 mL). After removal of the CH_3CN *in vacuo* the formed precipitate was filtered, washed with H_2O and dried *in vacuo* to afford the title compound **10** (179 mg, 22%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.82 (s, 1H), 8.69 (s, 1H), 8.34 (d, *J* = 7.5 Hz, 1H), 8.02 (s, 2H), 7.72 (d, *J* = 9.0 Hz, 2H), 7.46 (d, *J* = 8.0 Hz, 1H), 7.28 (br s, 2H), 7.23 – 7.16 (m, 2H), 6.83 (d, *J* = 9.0 Hz, 2H), 5.18 (s, 2H), 4.84 (s, 2H), 4.39 (ddd, *J* = 9.5, 7.5, 5.4 Hz, 1H), 3.61 (s, 3H), 3.56 (s, 3H), 3.20 (s, 3H), 2.44 – 2.37 (m, 5H), 2.13 – 1.88 (m, 2H).; ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.7, 172.6, 166.4, 162.0, 157.8, 155.1, 151.0,

150.7, 150.2, 147.9, 141.4, 136.2, 133.3, 129.0, 128.9, 124.3, 120.9, 120.8, 111.1, 66.7, 54.9, 51.8, 51.7, 51.3, 38.9, 29.9, 25.8, 22.1; HRMS (ESI) *m/z*: calcd for $C_{31}H_{36}BN_8O_9$ $[M+H]^+$ 675.2693, found 675.2720

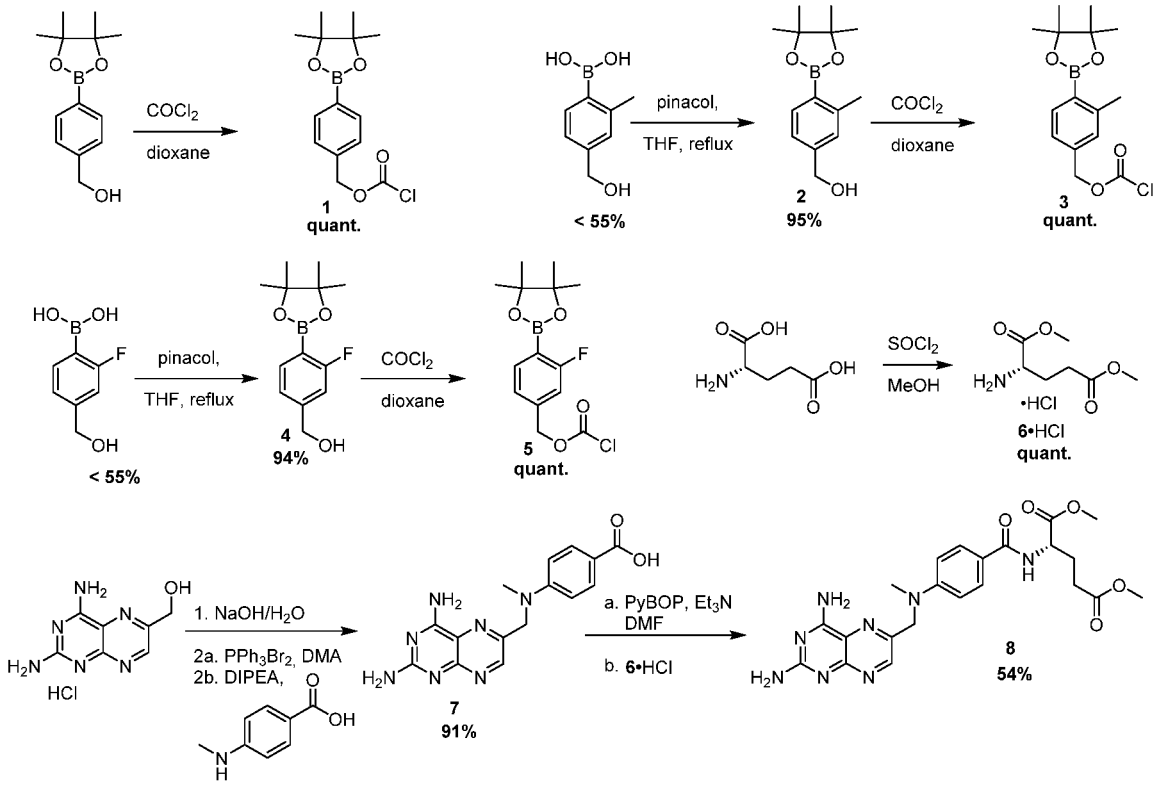
EXAMPLE 3

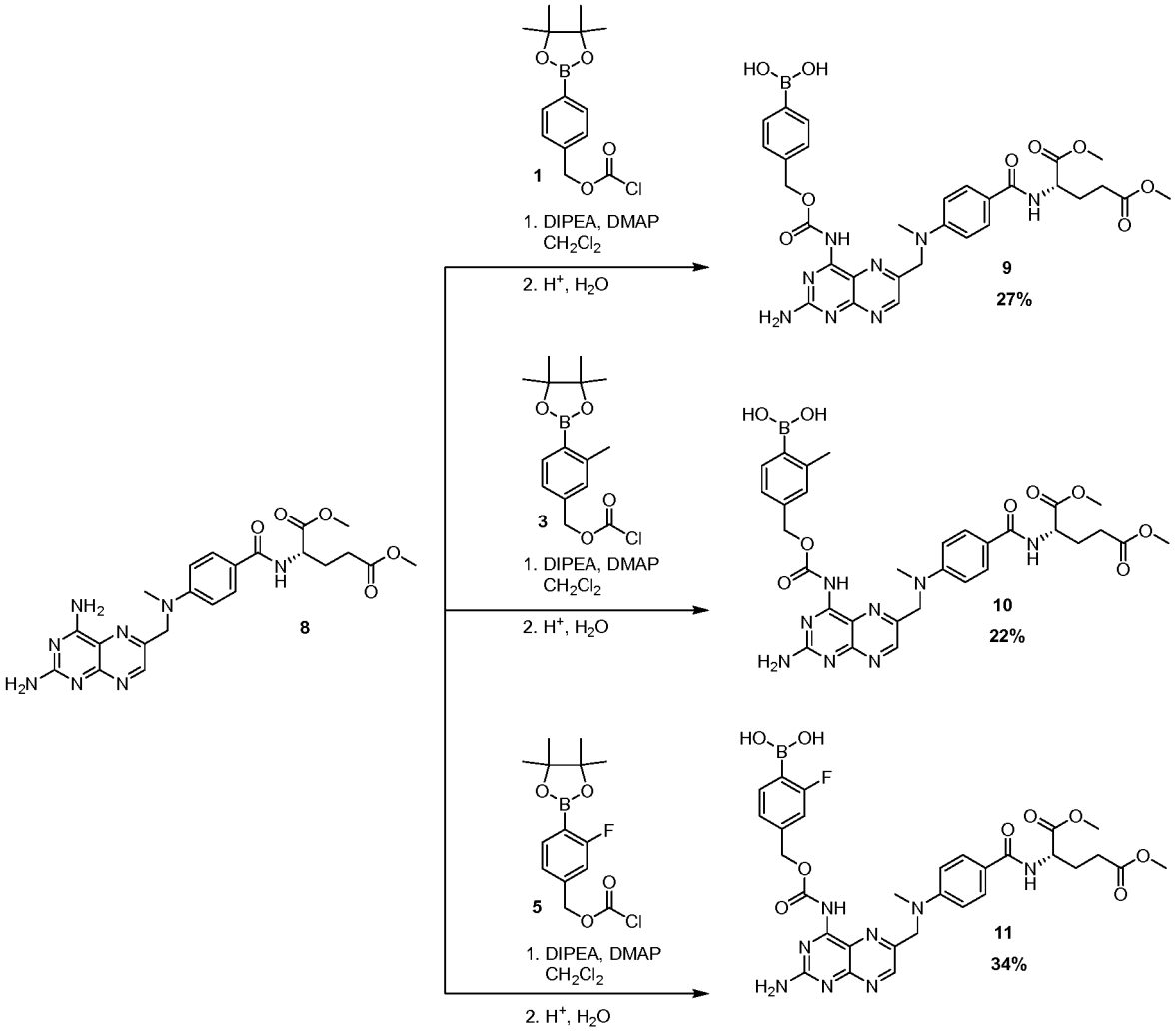
(S)-(4-((((2-Amino-6-(((4-((1,5-dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)carbamoyl)oxy)methyl)-2-fluorophenyl)boronic acid (11)



To a suspension of dimethyl (4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)benzoyl)-*L*-glutamate hydrochloride (**12**) (502 mg, 1.02 mmol) in dry CH_2Cl_2 (25 mL) was added DMAP (624 mg, 5.11 mmol) followed by DIPEA (0.89 mL, 5.11 mmol). The mixture was cooled to 0 °C and a solution of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2 yl)benzyl carbonochloridate (**5**) (1.30 g, 4.19 mmol) in CH_2Cl_2 (10 mL) was added dropwise. The reaction was allowed to warm to 21 °C and stirred for 5 h under a N_2 atmosphere. The mixture was diluted with CH_2Cl_2 (100 mL), washed with 1M HCl (2 x75 mL), sat. $NaHCO_3$ (2 x 75mL) and brine (75 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The solid residue was purified by preparative HPLC. The CH_3CN/H_2O fractions containing the pinacolate intermediate were poured together into a 250mL round bottom flask and HCl cc. (0.3 mL, ca. pH 2) was added. The reaction mixture was stirred for 16 h at room temperature and quenched with sat. $NaHCO_3$ (ca. 50 mL). After removal of the CH_3CN *in vacuo* the formed precipitate was filtered, washed with H_2O and dried *in vacuo* to afford **11** as a yellow solid (237 mg, 34%).^{1}H NMR (400 MHz, DMSO-d_6) δ 9.95 (s, 1H), 8.69 (s, 1H), 8.34 (d, *J* = 7.4 Hz, 1H), 8.22 (s, 2H), 7.72 (d, *J* = 8.8 Hz, 2H), 7.59 (t, *J* = 7.0 Hz, 1H), 7.24 (dd, *J* = 12.0, 9.0 Hz, 4H), 6.83 (d, *J* = 8.9 Hz, 2H), 5.25 (s, 2H), 4.85 (s, 2H), 4.39 (ddd, *J* = 9.4, 7.5, 5.7 Hz, 1H), 3.61 (s, 3H), 3.56 (s, 3H), 3.20 (s, 3H), 2.41 (t, *J* = 7.4 Hz, 2H), 2.16 – 1.86 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.2, 173.04, 166.9, 165.9 (d, *J* = 244.5 Hz), 162.5, 158.3, 155.6, 151.5, 151.1, 150.7, 148.4, 140.6 (d, *J* = 8.3 Hz), 136.0 (d, *J* = 9.8 Hz), 129.5, 123.4 (d, *J* = 2.7 Hz), 121.4, 121.3, 114.5 (d, *J* = 25.5 Hz), 111.6, 66.2,

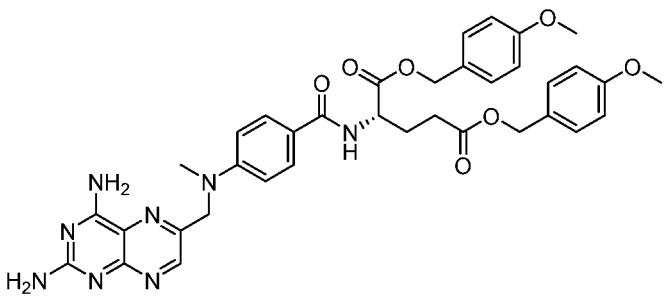
55.4, 52.3, 52.2, 51.8, 39.1, 30.4, 26.2; HRMS (ESI) *m/z*: calcd for $C_{30}H_{33}BFN_8O_9$ $[M+H]^+$ 679.2442, found 679.2455.




1
1. DIPEA, DMAP
CH2Cl2
2. H+, H2O
9
27%
3
1. DIPEA, DMAP
CH2Cl2
2. H+, H2O
10
22%
8
5
1. DIPEA, DMAP
CH2Cl2
2. H+, H2O
11
34%

PREPARATION EXAMPLE 9

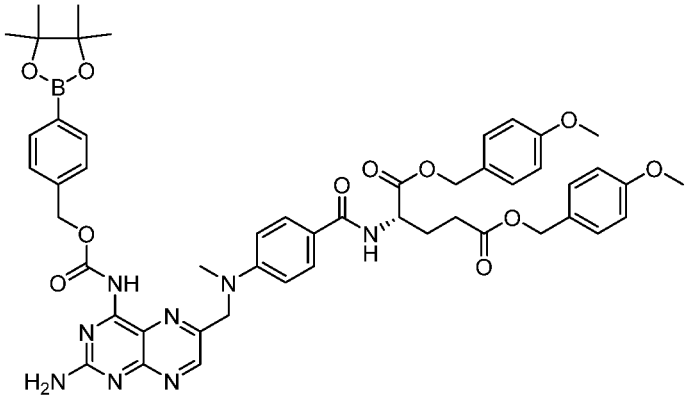
Bis(4-methoxybenzyl) (4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)benzoyl)-L-glutamate (13)



A solution of methotrexate (**12**) (1.0 g, 2.20 mmol) in anhydrous DMF (50 mL) was treated with 1,1,3,3-tetramethylguanidine (0.55 mL, 4.4 mmol) at 0 °C and the mixture was stirred for 30min under a N_2 atmosphere. Then 4-methoxybenzyl chloride (0.59 mL, 4.4 mmol) was added dropwise and the mixture was allowed to warm to 21 °C and stirred for 24 h. The volatiles were removed *in vacuo* and the resulting crude solid was purified by flash column chromatography on silica gel using a mixture of CH_2Cl_2/MeOH (from 97/3 to 94/6, v/v) as the eluent to give the title compound **13** (915 mg, 60%) as a yellow solid. Rf = 2.4 (silica, eluent CH_2Cl_2/MeOH, 95/5, v/v); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 (s, 1H), 8.34 (d, *J* = 7.5 Hz, 1H), 7.71 (d, *J* = 8.9 Hz, 2H), 7.65 (br s, 1H), 7.43 (br s, 1H), 7.27 (d, *J* = 6.9 Hz, 2H), 7.25 (d, *J* = 6.9 Hz, 2H), 6.90 – 6.86 (m, 4H), 6.82 (d, *J* = 8.9 Hz, 2H), 6.60 (br s, 2H), 5.10 – 4.99 (m, 2H), 4.97 (s, 2H), 4.78 (s, 2H), 4.42 (ddd, *J* = 9.6, 7.5, 5.4 Hz, 1H), 3.73 (s, 6H), 3.21 (s, 3H), 2.42 (t, *J* = 7.6 Hz, 2H), 2.13 – 1.90 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.1, 172.0, 166.5, 162.9, 162.7, 159.1, 155.2, 151.0, 149.2, 145.9, 129.8, 129.7, 129.0, 128.0, 127.9, 121.4, 120.8, 113.8, 111.0, 65.7, 65.3, 55.1, 54.8, 51.9, 38.8, 30.2, 25.8; HRMS (ESI) *m/z*: calcd for $C_{36}H_{39}N_8O_7$ $[M+H]^+$ 695.2936, found 695.2954.

EXAMPLE 4

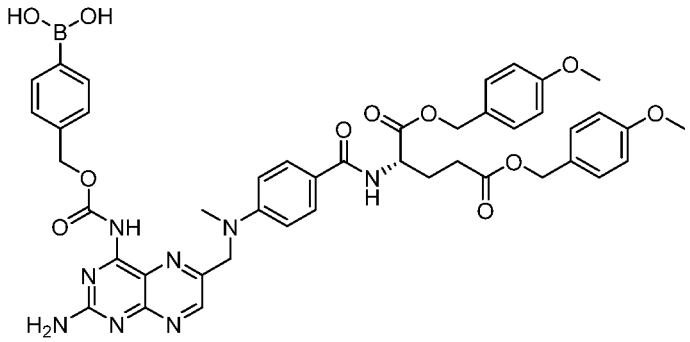
Bis(4-methoxybenzyl) (4-(((2-amino-4-((((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)carbonyl)amino)pteridin-6-yl)methyl)(methyl)amino)benzoyl)-L-glutamate (14)



To a suspension of bis(4-methoxybenzyl) (4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)benzoyl)-*L*-glutamate (**13**) (800 mg, 1.15 mmol) in anhydrous CH_2Cl_2 (25 mL) was added DMAP (703 mg, 5.76 mmol) followed by DIPEA (1.0 mL, 5.76 mmol). The mixture was cooled to 0 °C and a solution of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl carbonochloridate (**1**) (1.71 g, 5.76 mmol) in CH_2Cl_2 (10 mL) was added dropwise. The reaction was allowed to warm to 21 °C and stirred for 5 h under a N_2 atmosphere. Then the mixture was diluted with CH_2Cl_2 (100 mL), washed with 1M HCl (2 x75 mL), sat. $NaHCO_3$ (2 x 75mL), and brine (75 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification of the residue by preparative HPLC afforded the title compound **14** (800 mg, 73%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) 9.87 (s, 1H), 8.69 (s, 1H), 8.34 (d, *J* = 7.5 Hz, 1H), 7.72 (d, *J* = 7.9 Hz, 4H), 7.47 (d, *J* = 7.9 Hz, 2H), 7.33 – 7.17 (m, 6H), 6.91 – 6.85 (m, 4H), 6.82 (d, *J* = 9.0 Hz, 2H), 5.26 (s, 2H), 5.06 – 4.98 (m, 2H), 4.96 (s, 2H), 4.84 (s, 2H), 4.43 (ddd, *J* = 9.5, 7.4, 5.4 Hz, 1H), 3.72 (s, 6H), 3.19 (s, 3H), 2.41 (t, *J* = 7.4 Hz, 2H), 2.13 – 1.91 (m, 2H), 1.29 (s, 12H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.1, 172.0, 166.5, 162.0, 159.1, 157.8, 155.1, 151.1, 150.7, 150.3, 148.0, 139.3, 134.6, 129.8, 129.7, 129.0, 128.0, 127.9, 127.3, 121.0, 120.8, 113.8, 111.1, 83.7, 66.5, 65.7, 65.3, 55.1, 54.9, 51.9, 30.2, 25.8, 24.7; HRMS (ESI) *m/z*: calcd for $C_{50}H_{56}BN_8O_{11}$ $[M+H]^+$ 955.4156, found 955.4191.

EXAMPLE 5

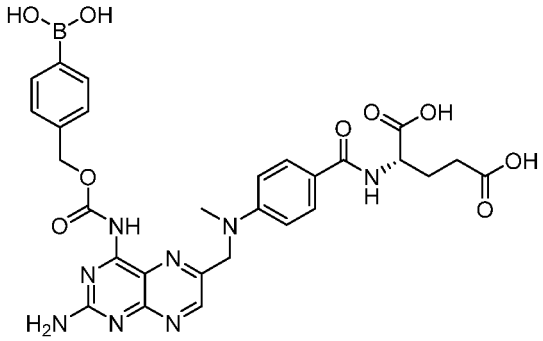
(S)-(4-((((2-Amino-6-(((4-((1,5-bis((4-methoxybenzyl)oxy)-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)carbamoyl)oxy)methyl)phenyl)boronic acid (15)



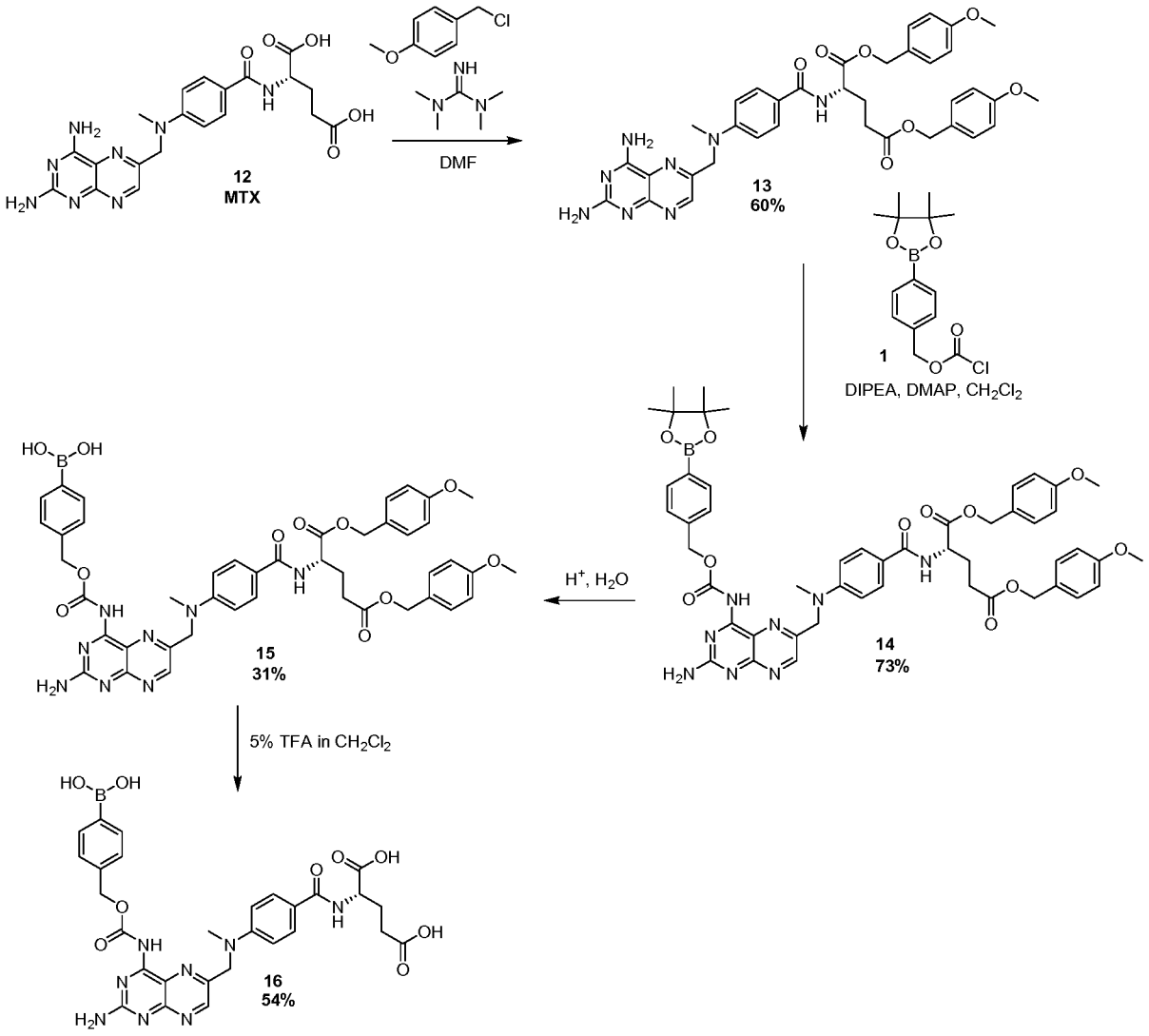
bis(4-methoxybenzyl) (4-(((2-amino-4-((((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)carbonyl)amino)pteridin-6-yl)methyl)(methyl)amino)benzoyl)-*L*-glutamate (**14**) (0.70 g, 0.73 mmol) was dissolved in a mixture of $CH_3CN/THF/H_2O$ (200mL, 2/1/1, v/v) and concentrated HCl was added until pH 2. The reaction mixture was stirred at 21 °C for 6 h followed by neutralization with sat. $NaHCO_3$ (ca. 50mL). After removal of the organic solvents *in vacuo*, the formed precipitate was filtered, washed with H_2O, and purified by preparative HPLC to afford the title compound **15** (199 mg, 31%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.87 (br s, 1H), 8.68 (s, 1H), 8.34 (d, *J* = 7.5 Hz, 1H), 8.25 (br s, 2H), 7.82 (d, *J* = 8.0 Hz, 2H), 7.72 (d, *J* = 8.9 Hz, 2H), 7.42 (d, *J* = 8.0 Hz, 2H), 7.30 – 7.17 (m, 6H), 6.92 – 6.84 (m, 5H), 6.82 (d, *J* = 8.9 Hz, 2H), 5.23 (s, 2H), 5.07 – 4.98 (m, 2H), 4.96 (s, 2H), 4.84 (s, 2H), 4.42 (ddd, *J* = 9.5, 7.5, 5.5 Hz, 1H), 3.72 (s, 6H), 3.19 (s, 3H), 2.41 (t, *J* = 7.7 Hz, 2H), 2.11 – 1.87 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.2, 172.0, 166.6, 162.1, 159.1, 157.8, 155.1, 151.1, 150.7, 150.3, 148.0, 137.7, 134.3, 129.9, 129.7, 129.1, 128.0, 127.9, 127.1, 121.0, 120.9, 113.8, 111.1, 66.7, 65.7, 65.4, 55.1, 54.9, 51.9, 39.06, 30.2, 25.8; HRMS (ESI) *m/z*: calcd for $C_{44}H_{46}BN_8O_{11}$ $[M+H]^+$ 873.3374, found 873.3405.

EXAMPLE 6

(4-(((2-Amino-4-((((4-boronobenzyl)oxy)carbonyl)amino)pteridin-6-yl)methyl)(methyl)amino)benzoyl)-L-glutamic acid (16)

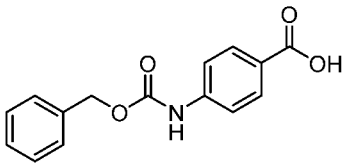


A stirred solution of (*S*)-(4-((((2-amino-6-(((4-((1,5-bis((4-methoxybenzyl)oxy)-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)carbamoyl)oxy)methyl)phenyl)boronic acid (**15**) (150 mg, 0.17mmol) in a 5% TFA solution in anhydrous CH_2Cl_2 (16 mL) was stirred for 25 min under a N_2 atmosphere. The mixture was concentrated *in vacuo* and the residue was purified by preparative HPLC to afford the title compound **16** as an orange solid (59 mg, 54%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.29 (br s, 2H), 9.89 (s, 1H), 8.68 (s, 1H), 8.20 (d, *J* = 7.7 Hz, 1H), 8.07 (s, 2H), 7.82 (d, *J* = 7.9 Hz, 2H), 7.73 (d, *J* = 8.9 Hz, 2H), 7.43 (d, *J* = 7.9 Hz, 2H), 7.28 (br s, 2H), 6.82 (d, *J* = 8.9 Hz, 2H), 5.24 (s, 2H), 4.84 (s, 2H), 4.34 (ddd, *J* = 9.7, 7.9, 5.0 Hz, 1H), 3.19 (s, 3H), 2.31 (t, *J* = 7.5 Hz, 2H), 2.11 – 1.99 (m, 1H), 1.99 – 1.79 (m, 1H);^{13}C NMR (101 MHz, DMSO-d_6) δ 173.9, 173.7, 166.3, 162.0, 157.8, 155.1, 151.0, 150.7, 150.3, 148.0, 137.7, 134.2, 129.0, 127.0, 121.3, 120.9, 111.1, 66.7, 54.9, 51.7, 39.0, 30.4, 26.0; HRMS (ESI) *m/z*: calcd for $C_{28}H_{28}BN_8O_9$ $[M-H]^-$ 631.2078, found 631.2028.


12
MTX
DMF
13
60%
1
DIPEA, DMAP, CH_2Cl_2
14
73%
H^+, H_2O
15
31%
5% TFA in CH_2Cl_2
16
54%

PREPARATION EXAMPLE 10

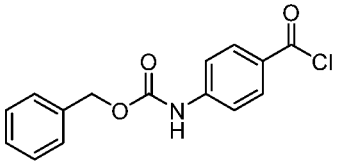
4-(((Benzyloxy)carbonyl)amino)benzoic acid (17)



To a solution of 4-methylaminobenzoic acid (4.11 g, 29.9 mmol) and $NaHCO_3$ (22.6 g, 269 mmol) in a mixture of H_2O/THF (100 mL, 1/1) was added benzyl chloroformate (4.26 mL, 29.9 mmol) dropwise at 0 °C. The reaction mixture was stirred for 4 h at 21 °C followed by addition of 20 mL of water. The mixture was stirred for additional 16 h followed by acidification with 1M HCl to pH 3. The precipitate was filtered and dried *in vacuo* to afford **17** (7.2 g, 89%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.66 (br s, 1H), 10.15 (s, 1H), 7.87 (d, *J* = 8.7 Hz, 2H), 7.58 (d, *J* = 8.7 Hz, 2H), 7.51 – 7.17 (m, 5H), 5.17 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.0, 153.2, 143.3, 136.3, 130.5, 128.5, 128.2, 128.2, 124.5, 117.3, 66.1.

PREPARATION EXAMPLE 11

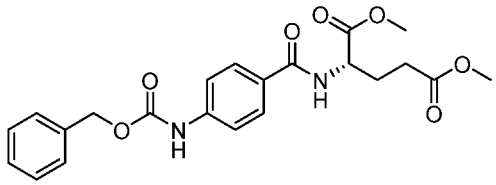
Benzyl (4-(chlorocarbonyl)phenyl)carbamate (18)



To a suspension of 4-(((benzyloxy)carbonyl)amino)benzoic acid (**17**) (2.90 g, 10.7 mmol) in anhydrous CH_2Cl_2 was added 1 drop of DMF followed by thionyl chloride (3.88 mL, 53.4 mmol) and the reaction mixture was refluxed under a N_2 atmosphere for 24h. All volatiles were removed *in vacuo* to afford the title compound **18** (3.1 g, quant.) as a white-pale yellow solid. The product was used for the next step within few hours without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ8.08 (d, *J* = 8.9 Hz, 2H), 7.54 (d, *J* = 8.9 Hz, 2H), 7.46 – 7.31 (m, 5H), 6.96 (br s, 1H), 5.23 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.4, 152.7, 144.5, 135.5, 133.4, 128.9, 128.8, 128.6, 127.8, 117.8, 67.8.

PREPARATION EXAMPLE 12

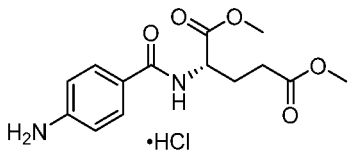
Dimethyl (4-(((benzyloxy)carbonyl)amino)benzoyl)-L-glutamate (19)



A mixture of dimethyl L-glutamate hydrochloride (**6**·HCl) (2.40 g, 11.3 mmol) and Et_3N in 100 mL of anhydrous CH_2Cl_2 was stirred under a N_2 atmosphere for 20 min at 21 °C. Then benzyl (4-(chlorocarbonyl)phenyl)carbamate (**18**) (3.0 g, 10.4 mmol) was added and the reaction was stirred for 2 h. The mixture was diluted with EtOAc, washed with 1M HCl (2 x 150 mL), sat. $NaHCO_3$ (2 x 150 mL), and brine (200 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford the title compound **19** (4.3 g, 97%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 8.6 Hz, 2H), 7.47 (d, *J* = 8.6 Hz, 2H), 7.44 – 7.29 (m, 5H), 6.97 (d, *J* = 10.4 Hz, 1H), 6.96 (s, 1H), 5.21 (s, 2H), 4.86 – 4.68 (m, 1H), 3.77 (s, 3H), 3.65 (s, 3H), 2.57 – 2.36 (m, 2H), 2.36 – 2.24 (m, 1H), 2.20 – 2.07 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.9, 172.6, 166.6, 153.07, 141.3, 135.9, 128.8, 128.6, 128.5, 128.5, 128.3, 118.0, 67.4, 52.8, 52.4, 52.1, 30.4, 27.3; HRMS (ESI) *m/z*: calcd for $C_{22}H_{25}N_2O_7$ $[M+H]^+$ 429.1656, found 429.1676.

PREPARATION EXAMPLE 13

Dimethyl (4-aminobenzoyl)-L-glutamate hydrochloride (20·HCl***)***

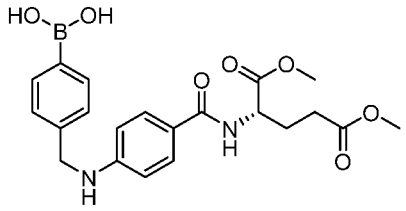


A solution of dimethyl (4-(((benzyloxy)carbonyl)amino)benzoyl)-L-glutamate (**19**) (2.0 g, 4.8 mmol) in dry MeOH (100 mL) was treated with 10% Pd/C (10% w/w, 200 mg). The mixture was stirred under a H2 atmosphere at 21 oC for 12 h, then filtered through a path of celite, concentrated in vacuo over silica gel and purified by flash column chromatography on silica gel using a mixture of EtOAc/Hep (2/1, v/v) as the eluent to give a clear oil corresponding to the free amine **20**. Treatment with 2M HCl in ether afforded the hydrochloride salt **20**·HCl (1.3 g, 95%) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ 7.88 (d, J

= 8.5 Hz, 2H), 7.42 (d, J = 8.5 Hz, 2H), 4.68 (dd, J = 9.4, 5.2 Hz, 1H), 3.79 (s, 3H), 3.65 (s, 3H), 2.59 (t, J = 7.0 Hz, 2H), 2.42 – 2.30 (m, 1H), 2.17 (ddt, J = 14.2, 9.4, 6.9 Hz, 1H); ^{13}C NMR (101 MHz, D_2O) δ 175.8, 173.7, 169.8, 136.8, 131.5, 129.2, 121.9, 53.0, 52.7, 52.3, 30.2, 25.5; HRMS (ESI) m/z: calcd for $C_{14}H_{19}N_2O_5$ $[M+H]^+$ 295.1288, found 295.1297.

PREPARATION EXAMPLE 14

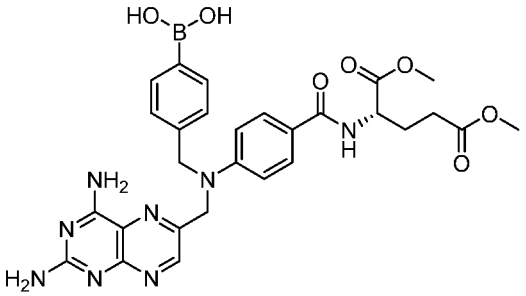
(S)-(4-(((4-((1,5-Dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)amino)methyl)phenyl)boronic acid (21)



To a solution of dimethyl (4-aminobenzoyl)-*L*-glutamate hydrochloride **20**.HCl (0.80 g, 2.42 mmol) in dry MeOH was added (4-formylphenyl)boronic acid (0.55 g, 3.67 mmol) followed by $NaBH_3CN$ (0.80 g, 12.1 mmol) in small portions. The reaction mixture was stirred under a N_2 atmosphere at 21 °C for 12 h. Extra (4-formylphenyl)boronic acid (0.55 g, 3.67 mmol) was added to the stirred mixture followed by additional 24 h of stirring for completion of the reaction. The crude mixture was concentrated in vacuo, dissolved in EtOAc (150mL), washed with sat. $NaHCO_3$ (2 x 150 mL), and brine (150 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford a pale yellow solid. Purification by preparative HPLC afforded the title compound **21** (699 mg, 67%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.24 (d, J = 7.5 Hz, 1H), 7.98 (s, 2H), 7.73 (d, J = 7.8 Hz, 2H), 7.62 (d, J = 8.6 Hz, 2H), 7.30 (d, J = 7.8 Hz, 2H), 6.84 (t, J = 6.0 Hz, 1H), 6.58 (d, J = 8.6 Hz, 2H), 4.38 (ddd, J = 9.5, 7.5, 5.6 Hz, 1H), 4.33 (d, J = 6.0 Hz, 2H), 3.61 (s, 3H), 3.57 (s, 3H), 2.41 (t, J = 7.4 Hz, 2H), 2.14 – 2.03 (m, 1H), 2.02 – 1.86 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.8, 172.7, 166.6, 151.4, 141.6, 134.2, 129.0, 126.2, 120.4, 111.1, 51.8, 51.7, 51.4, 46.0, 30.0, 25.8; HRMS (ESI) m/z: calcd for $C_{21}H_{26}BN_2O_7$ [M+H]+ 429.1828, found 429.1850.

EXAMPLE 7

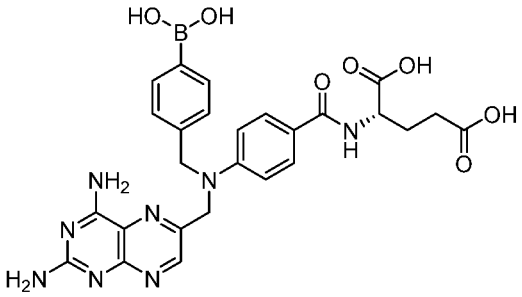
(S)-(4-((((2,4-Diaminopteridin-6-yl)methyl)(4-((1,5-dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)amino)methyl)phenyl)boronic acid (22)



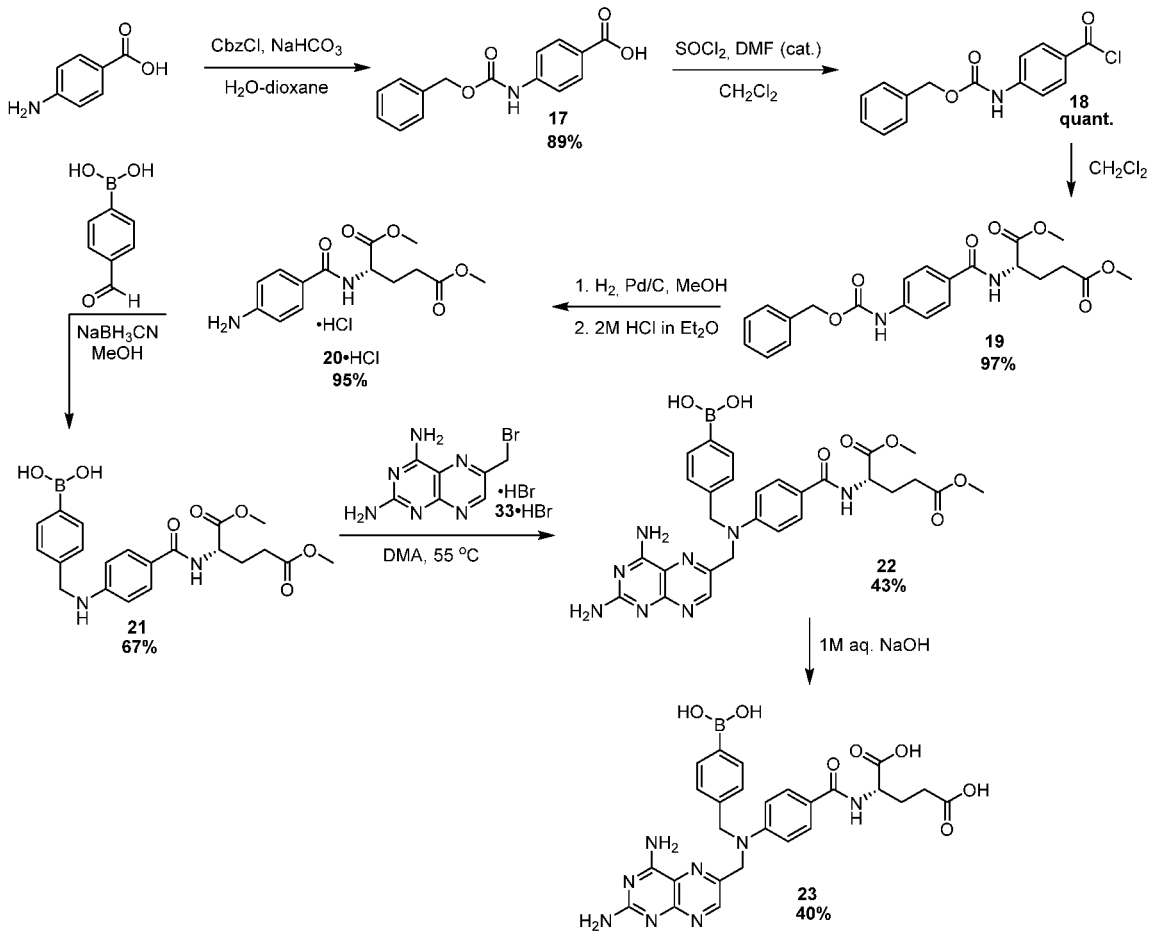
(*S*)-(4-(((4-((1,5-dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)amino)methyl)phenyl)boronic acid (**21**) (461 mg, 1.08 mmol) and 6-(bromomethyl)pteridine-2,4-diamine hydrobromide (515 mg, 1.38 mmol) were dissolved in dry DMA (4.85 mL) and the mixture was stirred at 55 °C for 3 days under a N_2 atmosphere. Then Et_3N (79 uL, 3.21 mmol) was added to the mixture followed by addition of H_2O. The orange precipitate was purified by preparative HPLC to afford **22** (279 mg, 43%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.63 (s, 1H), 8.30 (d, *J* = 7.5 Hz, 1H), 8.03 (br s, 2H), 7.74 (d, *J* = 7.9 Hz, 2H), 7.70 – 7.61 (m, 3H), 7.46 (br s, 1H), 7.23 (d, *J* = 7.9 Hz, 2H), 6.81 (d, *J* = 8.9 Hz, 2H), 6.62 (br s, 2H), 4.93 (s, 2H), 4.89 (s, 2H), 4.38 (ddd, *J* = 9.6, 7.5, 5.5 Hz, 1H), 3.60 (s, 3H), 3.56 (s, 3H), 2.40 (t, *J* = 7.4 Hz, 2H), 2.12 – 2.02 (m, 1H), 2.01 – 1.86 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.7, 172.5, 166.4, 162.9, 162.7, 155.3, 150.4, 149.4, 145.9, 140.4, 134.4, 128.9, 125.7, 121.4, 121.1, 111.5, 54.5, 54.0, 51.8, 51.7, 51.3, 29.9, 25.8; HRMS (ESI) *m/z*: calcd for $C_{28}H_{32}BN_8O_7$ $[M+H]^+$ 603.2482, found 603.2508.

EXAMPLE 8

(4-((4-Boronobenzyl)((2,4-diaminopteridin-6-yl)methyl)amino)benzoyl)-L-glutamic acid (23)

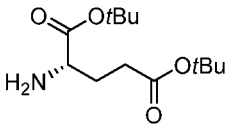


(*S*)-(4-((((2,4-diaminopteridin-6-yl)methyl)(4-((1,5-dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl) amino)methyl)phenyl)boronic acid **22** (46 mg, 76 μmol) was dissolved in 1M NaOH (2mL) and the mixture was stirred for 5 min. The crude was directly purified by preparative HPLC to afford the title compound **23** (17 mg, 40%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO) δ 8.63 (s, 1H), 8.08 (d, *J* = 7.3 Hz, 1H), 8.01 (s, 2H), 7.73 (d, *J* = 7.9 Hz, 2H), 7.69 – 7.55 (m, 3H), 7.46 (br s, 1H), 7.22 (d, *J* = 7.9 Hz, 2H), 6.81 (d, *J* = 8.9 Hz, 2H), 6.61 (br s, 2H), 4.91 (s, 2H), 4.88 (s, 2H), 4.36 – 4.25 (m, 1H), 2.36 – 2.18 (m, 2H), 2.02 – 1.78 (m, 2H). HRMS (ESI) *m/z*: calcd for $C_{26}H_{28}BN_8O_7$ $[M+H]^+$ 575.2169, found 575.2187



PREPARATION EXAMPLE 15

Di-tert-butyl L-glutamate (24)

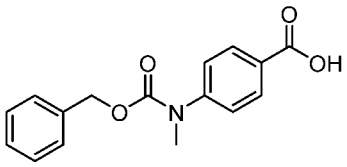


A suspension of L-glutamic acid (1.47 g, 10.0 mmol) in dry CH_2Cl_2 (40 mL) was placed in a pressure bottle and treated with concentrated H_2SO_4 (1.5 mL). The mixture was cooled to -78 oC followed by addition of condensed isobutylene (25 mL) within the pressure bottle at the same temperature. The bottle was closed and the reaction mixture was stirred at 21 oC for 5 days. Then the pressure bottle was cool to -78 oC and opened to dilute the reaction mixture with EtOAc (100 mL) followed by addition of sat. aq. $NaHCO_3$ (100 mL). The organic phase was separated and the aqueous phase washed with EtOAc (3 x 25 mL). The organic phases were collected, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford a clear oil. Purification by flash column chromatography on silica gel using a mixture of EtOAc/Hep (4/1,

v/v) as the eluent afforded the title compound **24** as a clear oil (1.26 g, 94%). Rf = 0.35 (silica, eluent EtOAc). ^{1}H NMR (400 MHz, C_6D_6) δ 3.17 (dd, J = 8.6, 4.9 Hz, 1H), 2.40 – 2.34 (m, 2H), 2.10 – 1.99 (m, 1H), 1.82 – 1.70 (m, 1H), 1.37 (s, 9H), 1.31 (s, 9H), 0.98 (br s, 1H), 0.44 (br s, 1H); 13C NMR (101 MHz, C_6D_6) δ 175.1, 172.4, 80.2, 79.6, 54.7, 32.1, 30.6, 28.1, 28.0.

PREPARATION EXAMPLE 16

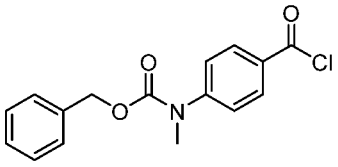
4-(((Benzyloxy)carbonyl)(methyl)amino)benzoic acid (25)



To a solution of 4-(methylamino)benzoic acid (3.03 g, 20.1 mmol) in anhydrous THF (20 mL) was added $NaHCO_3$ (2.05 g, 24.4 mmol) followed by benzyl chloroformate (1.70 mL, 12.0 mmol) dropwise at 0 °C. The reaction mixture was allowed to warm to 21 °C and was stirred for 2 h. Then the mixture was diluted with THF (20 mL), filtered, and concentrated *in vacuo*. The residue was dissolved in 1M NaOH (100 mL), washed with Et_2O (2 x 100 mL), acidified with 6M HCl to pH 3, and extracted with EtOAc (3 x 75 mL). The collected organic phases were dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford a pale orange solid. Recrystallization in EtOAc afforded the title compound **25** (4.49 g, 78%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, *J* = 8.8 Hz, 2H), 7.40 (d, *J* = 8.7 Hz, 2H), 7.38 – 7.28 (m, 5H), 5.21 (s, 2H), 3.39 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.0, 155.1, 148.22, 136.3, 131.1, 128.7, 128.3, 128.1, 126.1, 124.7, 67.9, 37.4.

PREPARATION EXAMPLE 17

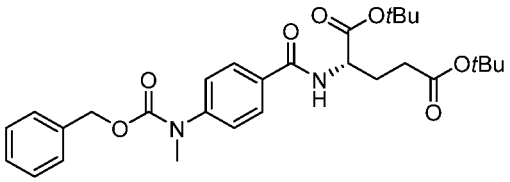
Benzyl (4-(chlorocarbonyl)phenyl)(methyl)carbamate (26)



A solution of 4-(((benzyloxy)carbonyl)(methyl)amino)benzoic acid (**25**) (1.0 g, 3.51 mmol) in anhydrous CH_2Cl_2 at 0 °C was treated with thionyl chloride (1.27 mL, 17.53 mmol). The mixture was allowed to warm to 21 °C and stirred for 24 h under a N_2 atmosphere. Concentration *in vacuo* afforded the title compound **26** (1.05 g, quant.) as a pale orange-pink solid. The acyl chloride **26** was used for the next step within few hours and without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, *J* = 8.7 Hz, 2H), 7.40 (d, *J* = 8.7 Hz, 2H), 7.38 – 7.28 (m, 5H), 5.21 (s, 2H), 3.39 (s, 3H).

PREPARATION EXAMPLE 18

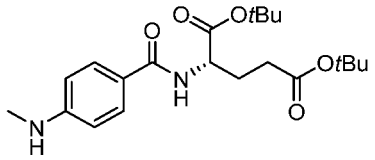
Di-tert-butyl (4-(((benzyloxy)carbonyl)(methyl)amino)benzoyl)-L-glutamate (27)



A solution of benzyl (4-(chlorocarbonyl)phenyl)(methyl)carbamate (**26**) (1.06 g, 3.49 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise to a stirred mixture of di-*tert*-butyl *L*-glutamate (**24**) (996 mg, 3.48 mmol) and Et_3N (0.97 mL, 6.98 mmol) in CH_2Cl_2 (15 mL). The reaction mixture was stirred at 21 °C under a N_2 atmoshphere for 2 h, then diluted with EtOAc (150 mL), washed with 1M HCl (2 x 100 mL), sat. aq. $NaHCO_3$ (2 x 100 mL), and brine (100 mL). The organic phase was dried over Na_2SO_4, and loaded in silica gel for purification by flash column chromatography on silica gel using a mixture of EtOAc/Hep (7/15, v/v) as the eluent to give the title compound **27** (1.75 g, 95%) as a thick orange oil. R_f = 0.53 (silica, eluent EtOAc/Hep, 1/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.7 Hz, 2H), 7.42 – 7.29 (m, 7H), 6.98 (d, *J* = 7.4 Hz, 1H), 5.18 (s, 2H), 4.75 – 4.53 (m, 1H), 3.35 (s, 3H), 2.53 – 2.13 (m, 3H), 2.12 – 1.95 (m, 1H), 1.49 (s, 9H), 1.41 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.8, 171.4, 166.4, 155.2, 146.3, 136.4, 131.3, 128.7, 128.3, 128.1, 127.9, 125.2, 82.6, 81.0, 67.8, 53.0, 37.6, 31.8, 28.2, 28.2, 27.6;

PREPARATION EXAMPLE 19

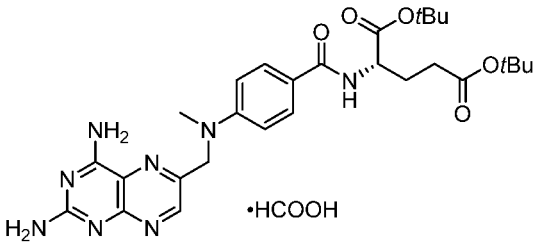
Di-tert-butyl (4-(methylamino)benzoyl)-L-glutamate (28)



To a solution of di-*tert*-butyl (4-(((benzyloxy)carbonyl)(methyl)amino)benzoyl)-L-glutamate (27) (1.35 g, 2.63 mmol) in dry MeOH (20 mL) was added 10% Pd/C (10% w/w, 135 mg) and the mixture was stirred for 3 days under a H_2 atmosphere at 21 °C. The crude mixture was filtered through a path of celite, concentrated in vacuo and purified by flash column chromatography on silica gel using a mixture of toluene/EtOAc/Et3N (8/2/0.1) as the eluent to afford 28 (895 mg, 87%) as an white solid. Rf = 0.24 (silica, eluent EtOAc/Hep, 3/7, v/v); ^{1}H NMR (400 MHz, CDCl3) δ 7.69 (d, J = 8.7 Hz, 2H), 6.72 (d, J = 7.5 Hz, 1H), 6.59 (d, J = 8.7 Hz, 2H), 4.72 – 4.57 (m, 1H), 4.36 (br s, 1H), 2.88 (s, 3H), 2.47 – 2.13 (m, 3H), 2.07 – 1.92 (m, 1H), 1.48 (s, 9H), 1.41 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.6, 171.7, 166.9, 151.8, 128.8, 122.2, 111.5, 82.2, 80.7, 52.6, 31.7, 30.4, 28.1, 28.0, 27.9; HRMS (ESI) m/z: calcd for $C_{21}H_{33}N_2O_5$ $[M+H]^+$ 393.2384, found 393.2430.

PREPARATION EXAMPLE 20

Di-tert-butyl (4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)benzoyl)-L-glutamate hydroformate (29·HCOOH)

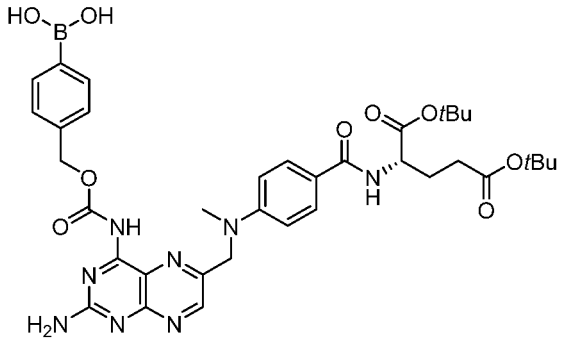


To a solution of 6-(bromomethyl)pteridine-2,4-diamine hydrobromide (0,72 g, 2.14 mmol) in dry DMAc (25 mL) was added di-*tert*-butyl (4-(methylamino)benzoyl)-L-glutamate (**28**) (0,76 g, 1.95 mmol) and the mixture was stirred for 24h at 55 °C under a N_2 atmosphere. The crude mixture was concentrated *in vacuo* and the dark residue was purified by preparative HPLC to afford the title compound **29**·HCOOH (430 mg, 36%) as an orange solid. ^{1}H NMR

(400 MHz, DMSO-d_6) δ 8.57 (s, 1H), 8.16 (d, *J* = 7.6 Hz, 1H), 8.14 (s, 1H), 7.72 (d, *J* = 9.0 Hz, 2H), 7.66 (br s, 1H), 7.44 (br s, 1H), 6.82 (d, *J* = 9.0 Hz, 2H), 6.61 (br s, 2H), 4.78 (s, 2H), 4.27 (ddd, *J* = 9.7, 7.5, 5.2 Hz, 1H), 3.21 (s, 3H), 2.29 (t, *J* = 7.6 Hz, 2H), 2.04 – 1.94 (m, 1H), 1.93 – 1.80 (m, 1H), 1.39 (s, 9H), 1.37 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.6, 171.4, 166.4, 162.9, 162.8, 162.7, 155.1, 150.9, 149.1, 146.0, 128.9, 121.4, 121.0, 111.0, 80.3, 79.7, 54.8, 52.3, 38.7, 31.4, 27.7, 27.6, 26.0; HRMS (ESI) *m/z*: calcd for $C_{28}H_{39}N_8O_5$ $[M+H]^+$ 567.3038, found 567.3055.

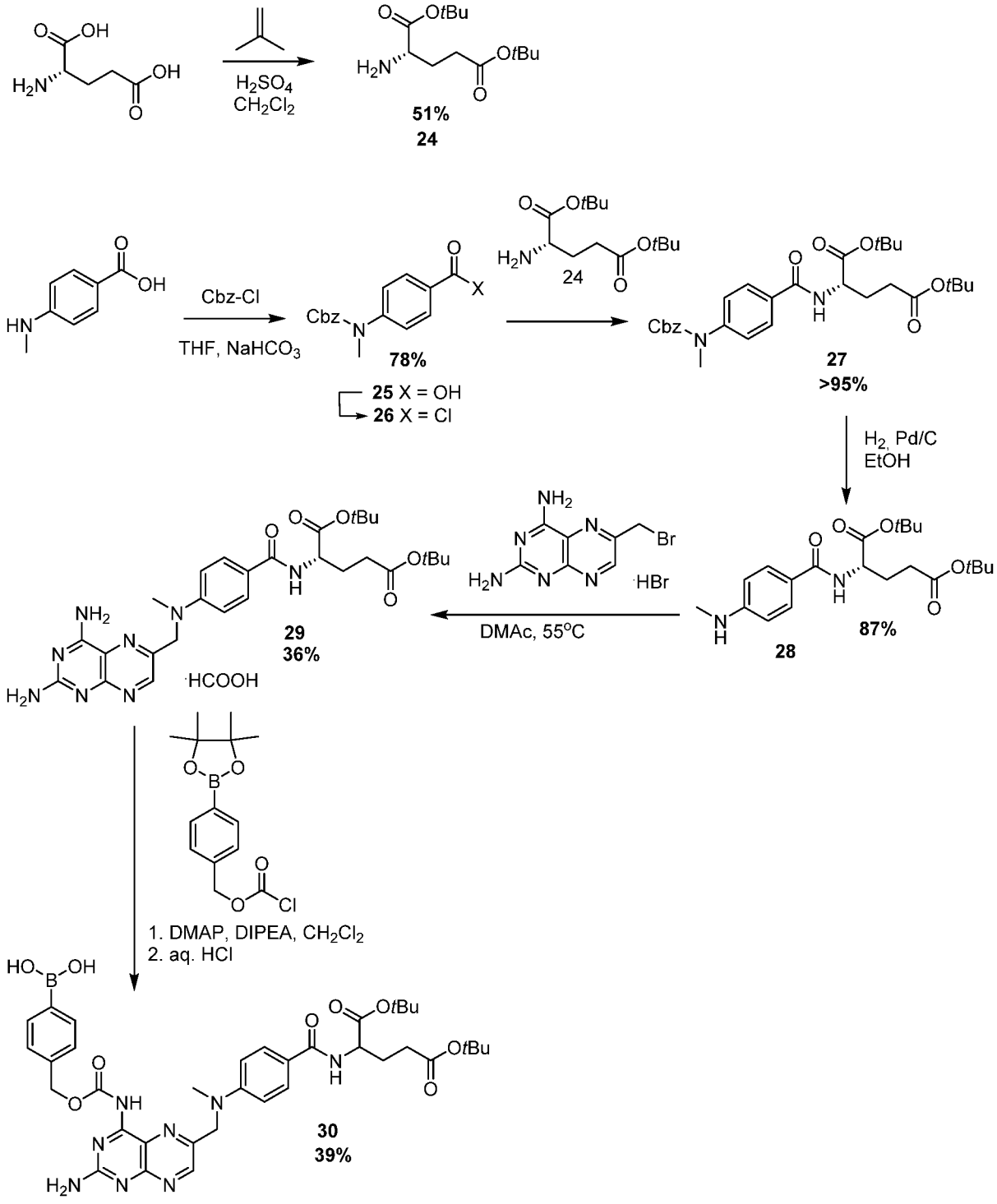
EXAMPLE 9

(S)-(4-((((2-Amino-6-(((4-((1,5-di-tert-butoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)carbamoyl)oxy)methyl)phenyl)boronic acid (30)



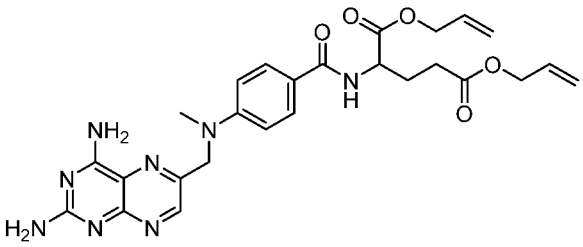
To a solution of di-*tert*-butyl (4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)benzoyl)-*L*-glutamate hydroformate **29**·HCOOH (280mg, 0.46 mmol) in anhydrous CH_2Cl_2 (15 mL) was added DMAP (279 mg, 2.29 mmol) followed by DIPEA (0.40 mL, 2.29 mmol). The mixture was cooled to 0 °C and a solution of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl carbonochloridate (**1**) (678 mg, 2.29 mmol) in CH_2Cl_2 (10 mL) was added dropwise. The reaction was allowed to warm to 21 °C and stirred for 4 h under a N_2 atmosphere. Then the crude mixture was concentrated *in vacuo* and the residue was purified by preparative HPLC. The CH_3CN/H_2O fractions containing the pinacolate intermediate were poured together into a 250 mL round bottom flask and concentrated HCl (0.3 mL, pH 2) was added. The reaction mixture was stirred for 16h at 21 °C and quenched with sat. $NaHCO_3$ (ca. 50 mL). After removal of CH_3CN *in vacuo*, the precipitate was filtered, washed with H_2O and dried *in vacuo* to afford **30** as a yellow solid (131 mg, 39%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.88 (s, 1H), 8.69 (s, 1H), 8.17 (d, *J* = 7.6 Hz, 1H), 8.07 (s, 2H), 7.82 (d, *J* = 7.8 Hz, 2H), 7.72 (d, *J* = 8.8 Hz, 2H), 7.43 (d, *J* = 7.8 Hz, 2H), 7.28 (br s, 2H), 6.82 (d, *J* = 8.8 Hz, 2H), 5.24 (s, 2H), 4.84 (s, 2H), 4.40 – 4.19 (m, 1H), 3.20 (s, 3H), 2.29 (t, *J* = 7.4 Hz, 2H), 2.04 – 1.93 (m, 1H), 1.93 – 1.82 (m, 1H), 1.38 (s, 9H), 1.36 (s, 9H); ^{13}C NMR

(101 MHz, DMSO-d_6) δ 171.6, 171.4, 166.4, 162.0, 157.8, 155.1, 151.0, 150.7, 150.3, 148.0, 137.7, 134.2, 129.0, 127.0, 121.2, 120.8, 111.1, 80.4, 79.7, 66.7, 54.9, 52.3, 38.7, 31.4, 27.7, 27.7, 26.0; HRMS (ESI) *m/z*: calcd for $C_{36}H_{46}BN_8O_9$ $[M+H]^+$ 745.3475, found 745.3493



PREPARATION EXAMPLE 21

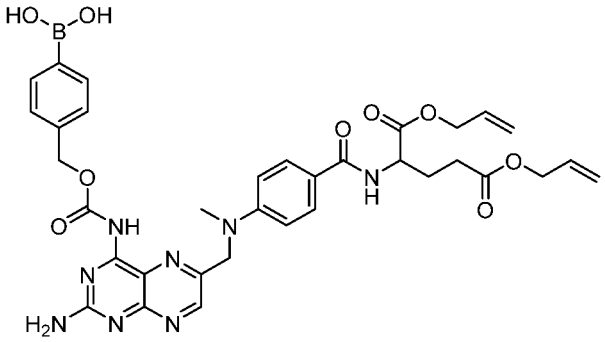
Diallyl (4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)benzoyl)glutamate (32)



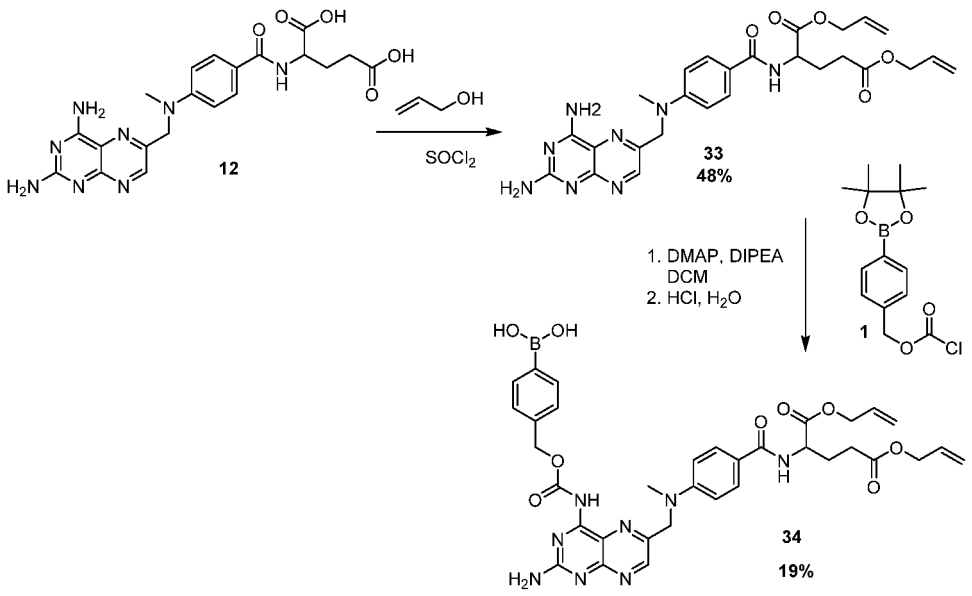
Thionyl chloride (6.38 mL, 88.0 mmol) was added dropwise at 0 °C to allyl alcohol (15 mL) and the mixture was stirred for 15 min at 21 °C. Then, methotrexate (**12**) (800 mg, 1.76 mmol) was added to the mixture and the reaction was stirred for 2 days under a N_2 atmosphere. The crude mixture was then concentrated *in vacuo* and purified by column chromatography on reverse phase silica gel using a mixture of H_2O/CH_3CN (from 0% CH_3CN to 80%) to afford the title compound **31** (450 mg, 48%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 (s, 1H), 8.36 (d, *J* = 7.4 Hz, 1H), 7.88 (br s, 1H), 7.73 (d, *J* = 8.9 Hz, 2H), 7.66 (br s, 1H), 6.92 – 6.68 (m, 4H), 5.96 – 5.73 (m, 2H), 5.34 – 5.23 (m, 2H), 5.21 – 5.13 (m, 2H), 4.79 (s, 2H), 4.60 – 4.55 (m, 2H), 4.53 (d, *J* = 5.4 Hz, 2H), 4.43 (ddd, *J* = 9.6, 7.4, 5.3 Hz, 1H), 3.22 (s, 3H), 2.50 – 2.42 (m, 2H), 2.18 – 1.84 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.4, 172.2, 167.0, 163.2, 162.3, 154.3, 151.5, 149.6, 147.1, 133.1, 132.9, 129.5, 122.0, 121.3, 118.2, 118.0, 111.5, 65.2, 64.9, 55.3, 52.3, 38.9, 30.6, 26.2; HRMS (ESI) *m/z*: calcd for $C_{26}H_{31}N_8O_5$ $[M+H]^+$ 535.2412, found 535.2436.

EXAMPLE 10

(4-((((2-Amino-6-(((4-((1,5-bis(allyloxy)-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)carbamoyl)oxy)methyl)phenyl)boronic acid (33)

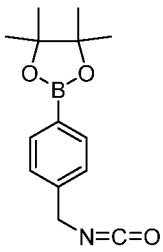


To a suspension of diallyl (4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)benzoyl)-glutamate (**31**) (320 mg, 0.60 mmol) in dry CH_2Cl_2 (15 mL) was added DMAP (366 mg, 2.99 mmol) followed by DIPEA (0.521 mL, 2.99 mmol). The mixture was cooled to 0 °C and a solution of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl carbonochloridate **1** (1.07 g, 3.59 mmol) in CH_2Cl_2 (7 mL) was added dropwise. The reaction was allowed to warm to 21 °C and stirred for 5 h under a N_2 atmosphere. The crude mixture was concentrated *in vacuo* to afford a dark yellow solid. The solid residue was purified by preparative HPLC. The CH_3CN/H_2O fractions containing the pinacolate intermediate were poured together into a 250mL round bottom flask and HCl cc. (0.3 mL, ca. pH 2) was added. The reaction mixture was stirred for 16h at room temperature and quenched with excess of sat. $NaHCO_3$ (ca. 50 mL). After removal of CH_3CN *in vacuo* a yellow solid was filtered, washed with H_2O and dried *in vacuo* to afford **36** (82 mg, 19%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.88 (s, 1H), 8.69 (s, 1H), 8.36 (d, *J* = 7.5 Hz, 1H), 8.07 (s, 2H), 7.82 (d, *J* = 7.9 Hz, 2H), 7.72 (d, *J* = 8.9 Hz, 2H), 7.43 (d, *J* = 7.9 Hz, 2H), 7.28 (br s, 2H), 6.83 (d, *J* = 9.0 Hz, 2H), 5.99 – 5.73 (m, 2H), 5.35 – 5.26 (m, 2H), 5.24 (s, 2H), 5.21 – 5.13 (m, 2H), 4.84 (s, 2H), 4.61 – 4.45 (m, 4H), 4.43 (ddd, *J* = 9.7, 7.5, 5.4 Hz, 1H), 3.20 (s, 3H), 2.46 (t, *J* = 7.5 Hz, 2H), 2.18 – 1.84 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.9, 171.7, 166.5, 162.0, 157.8, 155.1, 151.0, 150.7, 150.3, 147.9, 137.7, 134.2, 132.6, 132.5, 129.0, 127.0, 120.9, 120.8, 117.7, 117.6, 111.1, 66.6, 64.7, 64.4, 54.9, 51.9, 38.7, 30.1, 25.7. HRMS (ESI) *m/z*: calcd for $C_{34}H_{38}BN_8O_9$ $[M+H]^+$ 713,2849, found 713.2883


12
SOCl2
33
48%
1. DMAP, DIPEA
DCM
2. HCl, H2O
1
34
19%

PREPARATION EXAMPLE 22

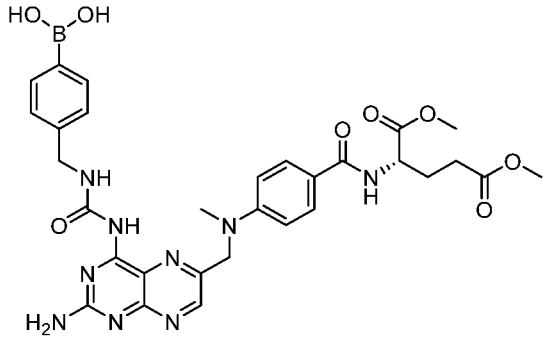
2-(4-(Isocyanatomethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (34)



To a suspension of (4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)methanamine hydrochloride (148 mg, 0.5 mmol) in anhydrous dioxane (3.0 mL) was added Et_3N (0.2 mL, 1.4 mmol). After stirring for 30 min, $COCl_2$ (2.0 mL, 15% in toluene) was added and the mixture was stirred for 16 h under a N_2 atmosphere at 21 °C. Next, the reaction was diluted with toluene (10 mL), filtered, and concentrated *in vacuo* to afford the title compound **34** (138 mg, >95%), which was used for next step without further purification.

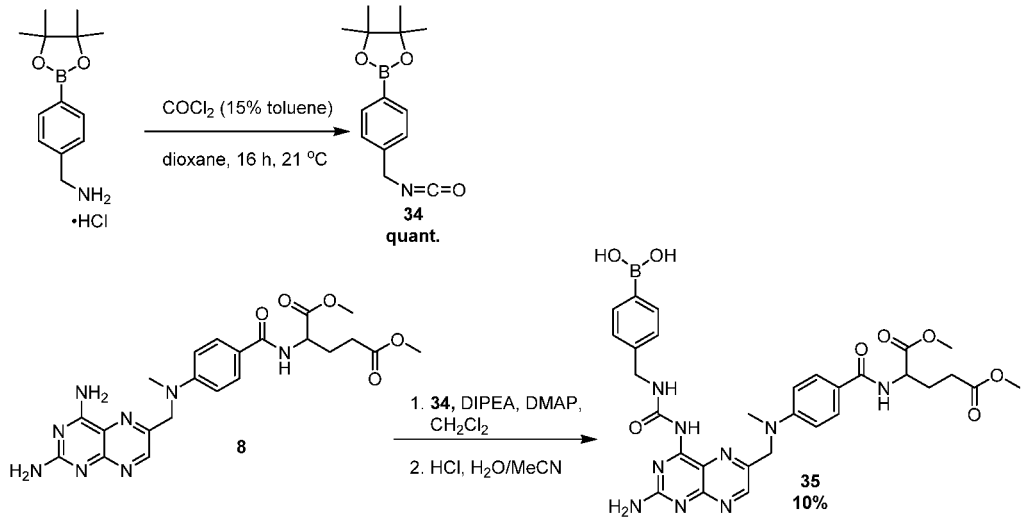
EXAMPLE 11

(*S*)-(4-((3-(2-Amino-6-(((4-((1,5-dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)ureido)methyl)phenyl)boronic acid (35)



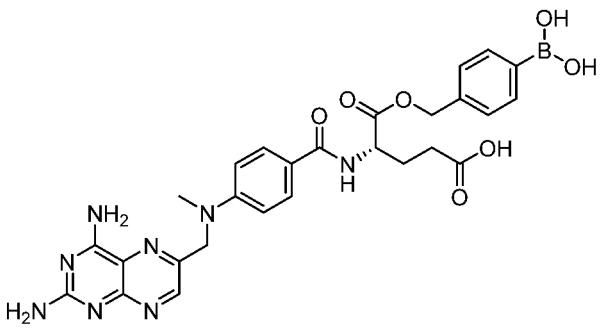
To a solution of dimethyl (4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino) benzoyl)-*L*-glutamate **8** (100 mg, 0.2 mmol) in CH_2Cl_2 (5.0 mL) was added DIPEA (72 µL, 0.4 mmol) and DMAP (51 mg, 0.4 mmol), followed by dropwise addition of a solution of 2-(4-(isocyanatomethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane **34** (107 mg, 0.4 mmol) in CH_2Cl_2 at 0 °C. The reaction was allowed to warm to 21 °C and stirred for 16 h under a N_2 atmosphere. The mixture was diluted with CH_2Cl_2 (100 mL), washed with 1 M aq. HCl (2 x75

mL), sat. aq. $NaHCO_3$ (2 x 75mL), and brine (75 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford a solid that was purified by preparative HPLC. The CH_3CN/H_2O fractions containing the pinacolate intermediate were poured together and HCl cc. was added until pH ~ 2. The reaction mixture was stirred for 16 h at 21 °C and quenched with sat. aq. $NaHCO_3$ (50 mL). After removal of the CH_3CN *in vacuo* the formed precipitate was filtered, washed with H_2O and dried *in vacuo* to afford the title compound **35** (13 mg, 10%) as a yellow solid. UPLC-MS (method A): R_t = 1.7 min; MS (ESI^+) m/z: calcd for $C_{30}H_{35}BN_9O_8$ $[M+H]^+$ 660.3, found 660.3; MS (ESI^-) m/z: calcd for $C_{30}H_{33}BN_9O_8$ $[M-H]^-$ 658.3, found 658.2.



EXAMPLE 12

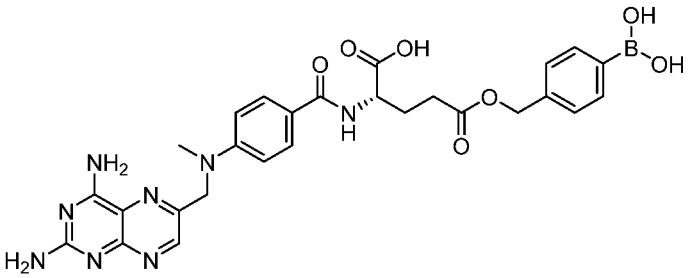
(S)-5-((4-Boronobenzyl)oxy)-4-(4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)benzamido)-5-oxopentanoic acid (37)



To a solution of methotrexate **12** (100 mg, 0.2 mmol) in dry DMF (5.0 mL) was added 1,1,3,3-tetramethylguanidine (82 µL, 0.7 mmol) followed by (4-(bromomethyl)phenyl)boronic acid (47 mg, 0.2 mmol) and the reaction mixture was stirred at 50 °C for 3 days. The crude mixture was then purified by preparative HPLC to afford the title compound **37** as a yellow solid (15 mg, 12%). MS (ESI^+) m/z: calcd for $C_{27}H_{30}BN_8O_7$ $[M+H]^+$ 589.2, found 589.4; MS (ESI^-) m/z: calcd for $C_{27}H_{28}BN_8O_7$ $[M-H]^-$ 587.2, found 587.2.

EXAMPLE 13

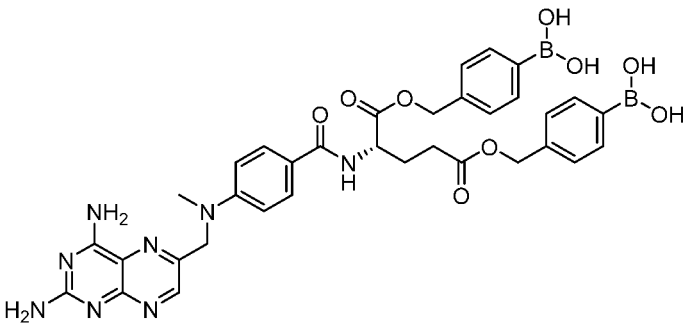
(S)-5-((4-Boronobenzyl)oxy)-2-(4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)benzamido)-5-oxopentanoic acid (38)



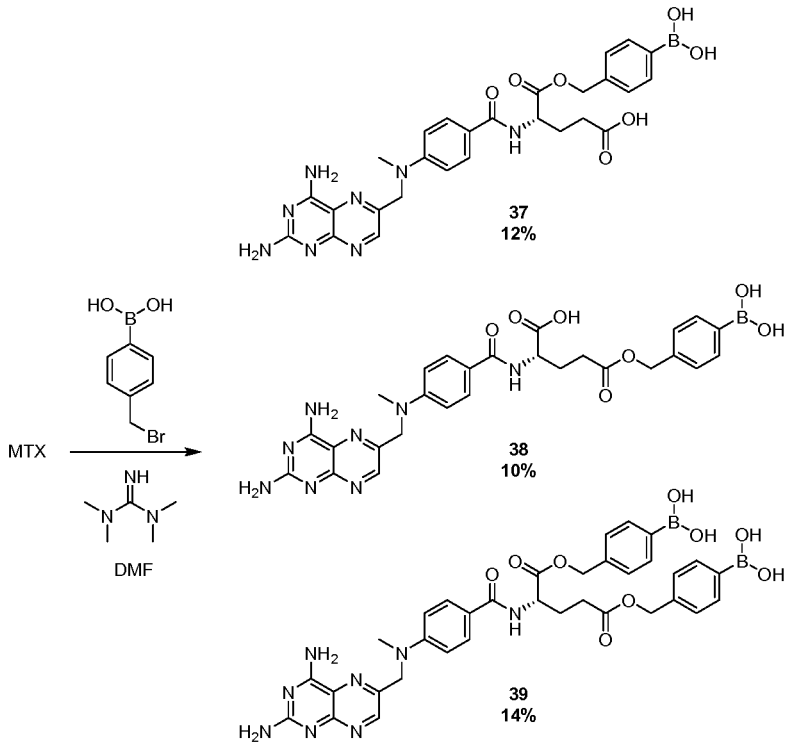
To a solution of methotrexate **12** (100 mg, 0.2 mmol) in dry DMF (5.0 mL) was added 1,1,3,3-tetramethylguanidine (82 µL, 0.7 mmol) followed by (4-(bromomethyl)phenyl)boronic acid (47 mg, 0.2 mmol) and the reaction mixture was stirred at 50 °C for 3 days. The crude mixture was then urified by preparative HPLC to afford the title compound **38** as a yellow solid (13 mg, 10%). MS (ESI^+) m/z: calcd for $C_{27}H_{30}BN_8O_7$ $[M+H]^+$ 589.2, found 589.4; MS (ESI^-) m/z: calcd for $C_{27}H_{28}BN_8O_7$ $[M-H]^-$ 587.2, found 587.2.

EXAMPLE 14

(*S*)-(4-(((5-((4-Boronobenzyl)oxy)-2-(4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)benzamido)-5-oxopentanoyl)oxy)methyl)phenyl)boronic acid (39)

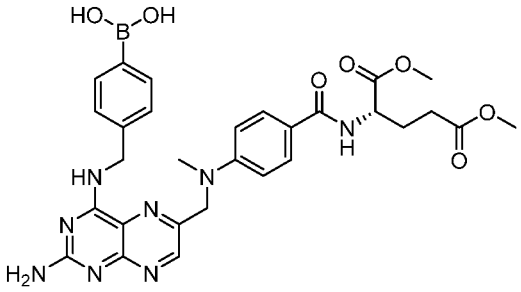


To a solution of methotrexate **12** (100 mg, 0.2 mmol) in dry DMF (5.0 mL) was added 1,1,3,3-tetramethylguanidine (82 µL, 0.7 mmol) followed by (4-(bromomethyl)phenyl)boronic acid (94 mg, 0.4 mmol) and the reaction mixture was stirred at 50 °C for 3 days. The crude mixture was then purified by preparative HPLC to afford the title compound **39** as a yellow solid (22 mg, 14%). MS (ESI^+) m/z: calcd for $C_{34}H_{37}B_2N_8O_9$ $[M+H]^+$ 723.3, found 723.3.



EXAMPLE 15

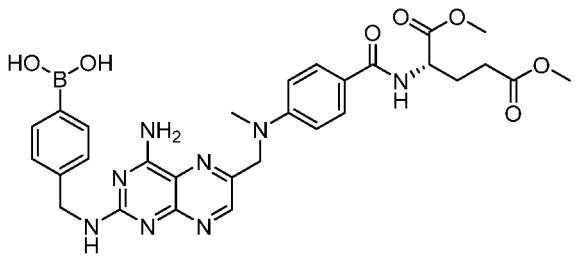
(*S*)-(4-(((2-Amino-6-(((4-((1,5-dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)amino)methyl)phenyl)boronic acid (40)



To a solution of (4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)benzoyl)-*L*-glutamate hydrochloride **8** (100 mg, 0.2 mmol) in dry DMF (5.0 mL) was added K_2CO_3 (86 mg, 0.6 mmol) followed by (4-(bromomethyl)phenyl)boronic acid (44 mg, 0.2 mmol) and the reaction mixture was stirred at 90 °C for 60 min. The crude was then purified by preparative HPLC to afford the title compound **40** (10 mg, 8%) as a solid. MS (ESI^+) m/z: calcd for $C_{29}H_{34}BN_8O_7$ $[M+H]^+$ 617.3, found 617.2; MS (ESI^-) m/z: calcd for $C_{29}H_{32}BN_8O_7$ $[M-H]^-$ 615.2, found 615.2.

EXAMPLE 16

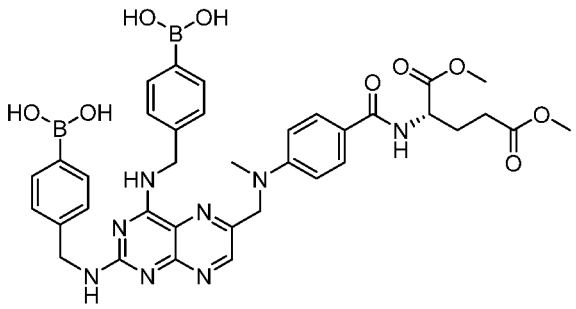
(*S*)-(4-(((4-Amino-6-(((4-((1,5-dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-2-yl)amino)methyl)phenyl)boronic acid (41)



To a solution of (4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)benzoyl)-*L*-glutamate hydrochloride **8** (100 mg, 0.2 mmol) in dry DMF (5.0 mL) was added K_2CO_3 (86 mg, 0.6 mmol) followed by (4-(bromomethyl)phenyl)boronic acid (44 mg, 0.2 mmol) and the reaction mixture was stirred at 90 °C for 60 min. The crude was then purified by preparative HPLC to afford the title compound **41** (6 mg, 5%) as a solid. MS (ESI^+) m/z: calcd for $C_{29}H_{34}BN_8O_7$ $[M+H]^+$ 617.3, found 617.2; MS (ESI^-) m/z: calcd for $C_{29}H_{32}BN_8O_7$ $[M-H]^-$ 615.2, found 615.2.

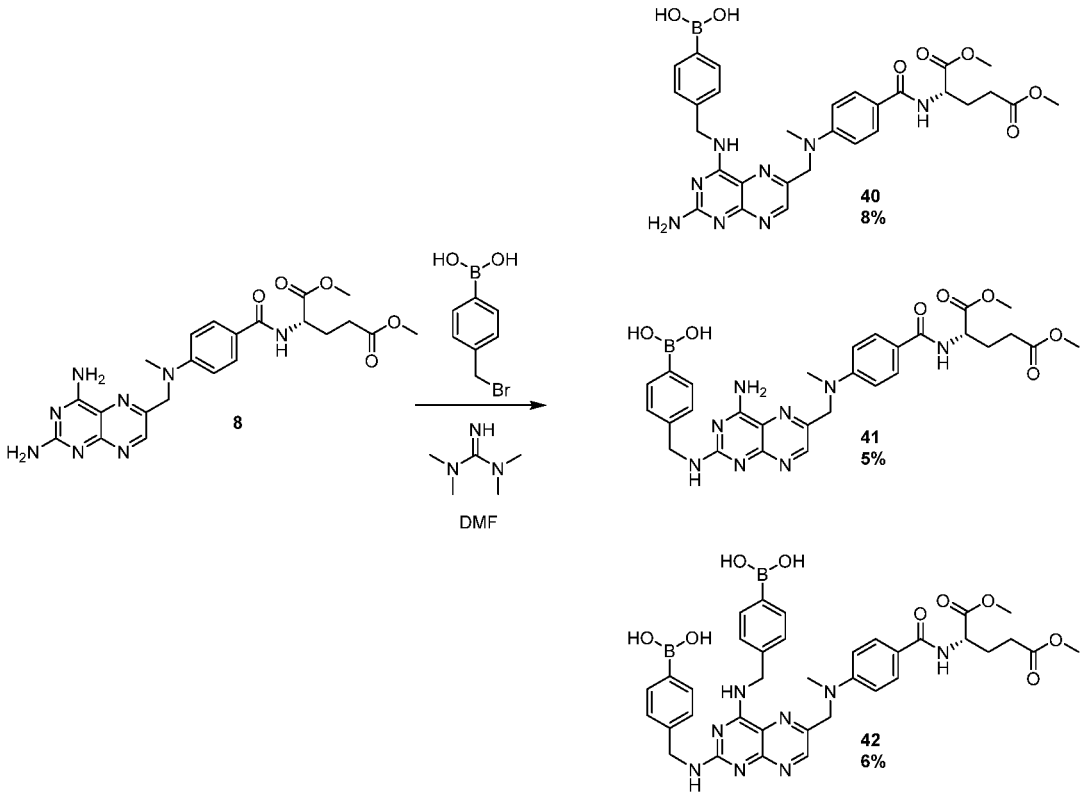
EXAMPLE 17

(*S*)-((((6-(((4-((1,5-Dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridine-2,4-diyl)bis(azanediyl))bis(methylene))bis(4,1-phenylene))diboronic acid (42)



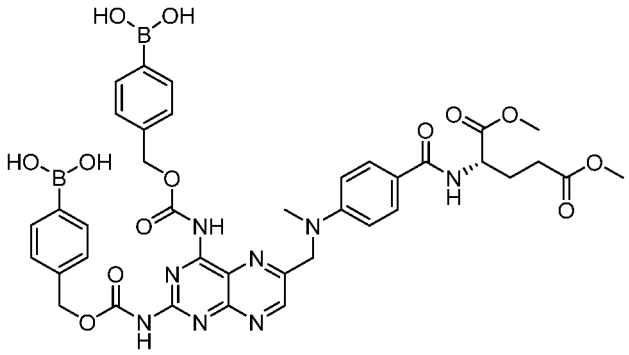
To a solution of **8** (100 mg, 0.2 mmol) in dry DMF (5.0 mL) was added K_2CO_3 (86 mg, 0.6 mmol) followed by (4-(bromomethyl)phenyl)boronic acid (88 mg, 0.4 mmol) and the reaction mixture was stirred at 90 °C for 60 min. The crude was then purified by preparative HPLC to

afford the title compound **42** (9 mg, 6%) as a solid. MS (ESI^+) m/z: calcd for $C_{36}H_{41}B_2N_8O_9$ $[M+H]^+$ 751.3, found 751.3; MS (ESI^-) m/z: calcd for $C_{36}H_{39}B_2N_8O_9$ $[M-H]^-$ 749.2, found 749.3.

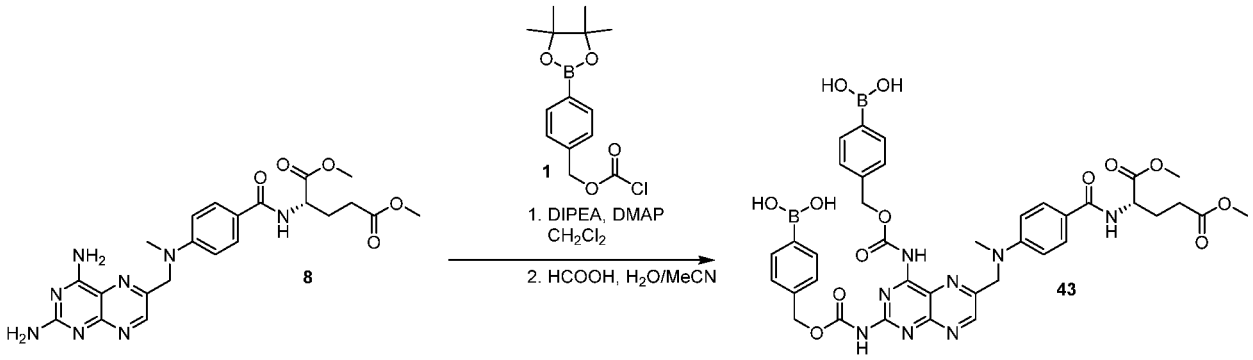


EXAMPLE 18

(*S*)-((((((6-(((4-((1,5-Dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridine-2,4-diyl)bis(azanediyl))bis(carbonyl))bis(oxy))bis(methylene))bis(4,1-phenylene))diboronic acid (43)

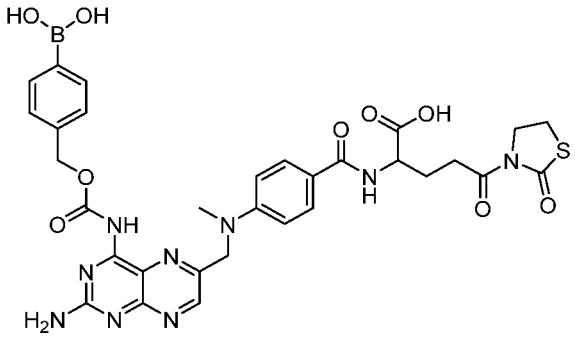


To a suspension of dimethyl (4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)benzoyl)-*L*-glutamate hydrochloride **8** (313 mg, 0.68 mmol) in dry CH_2Cl_2 (25 mL) was added DMAP (417 mg, 3.42 mmol) followed by DIPEA (0.60 mL, 3.42 mmol). The mixture was cooled to 0 °C and a solution of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl carbonochloridate (**1**) (1.01 g, 3.42 mmol) in CH_2Cl_2 (10 mL) was added dropwise. The reaction was allowed to warm to 21 °C and stirred for 5 h under a N_2 atmosphere. The mixture was then diluted with CH_2Cl_2 (100 mL), washed with 1 M aq. HCl (2 x75 mL), sat. aq. $NaHCO_3$ (2 x 75mL), and brine (75 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford a yellow solid that was purified by preparative HPLC. The CH_3CN/H_2O fractions containing the pinacolate intermediate were poured together and HCl cc. was added until pH ~2 was added. The reaction mixture was stirred for 16 h at 21 °C and quenched with sat. aq. $NaHCO_3$ (50 mL). After removal of the CH_3CN *in vacuo* the formed precipitate was filtered, washed with H_2O and dried *in vacuo* to afford the title compound **43** (15 mg, 3%) as a yellow solid. ^1H NMR (400 MHz, DMSO) δ 10.77 (br s, 1H), 10.33 (br s, 1H), 8.94 (s, 1H), 8.35 (d, *J* = 7.4 Hz, 2H), 8.09 (s, 2H), 8.06 (s, 2H), 7.82 (d, *J* = 7.9 Hz, 2H), 7.79 (d, *J* = 8.1 Hz, 2H), 7.72 (d, *J* = 8.8 Hz, 2H), 7.44 (d, *J* = 8.1 Hz, 2H), 7.41 (d, *J* = 8.0 Hz, 2H), 6.84 (d, *J* = 8.9 Hz, 2H), 5.26 (s, 2H), 5.20 (s, 2H), 4.95 (s, 2H), 4.49 – 4.28 (m, 1H), 3.60 (s, 3H), 3.56 (s, 3H), 3.23 (s, 3H), 2.40 (t, *J* = 7.4 Hz, 2H), 2.17 – 1.80 (m, 1H). RP-UPLC-MS (method A): R_t = 1.34 min; MS (ESI^+) m/z: calcd for $C_{38}H_{41}B_2N_8O_{13}$ $[M+H]^+$ 839.3, found 839.2; MS (ESI^-) m/z: calcd for $C_{38}H_{39}B_2N_8O_{13}$ $[M-H]^-$ 837.3, found 837.3.



EXAMPLE 19

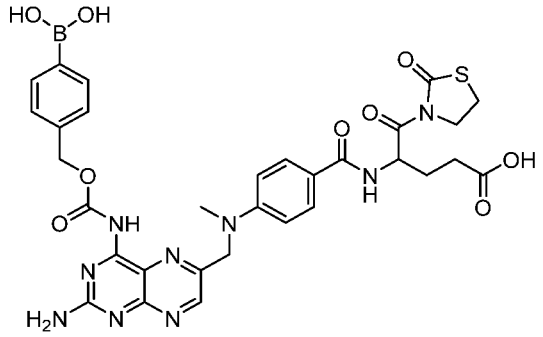
(2-(4-(((2-Amino-4-((((4-boronobenzyl)oxy)carbonyl)amino)pteridin-6-yl)methyl)(methyl)amino)benzamido)-5-oxo-5-(2-oxothiazolidin-3-yl)pentanoic acid (44)



To a solution of **16** (100 mg, 0.16 mmol) in dry DMF (5 mL) was added DCC (55 mg, 0.27 mmol) followed by DMAP (58 mg, 0.47 mmol). The reaction mixture was stirred for 15 min under a N_2 atmosphere at 21 °C followed by addition of thiazolidin-2-one (28 mg, 0.27 mmol) and additional stirring for 16 h. Filtration of the crude reaction and concentration of the filtrate *in vacuo* afforded a residue which was purified by preparative HPLC to give the title compound **44** as a solid (4.5 mg, 4%). MS (ESI^+) m/z: calcd for $C_{31}H_{32}BN_9O_8S$ $[M+H-H_2O]^+$ 700.5, found 699.9; MS (ESI^-) m/z: calcd for $C_{31}H_{31}BN_9O_9S$ $[M-H]^-$ 716.2, found 716.3.

EXAMPLE 20

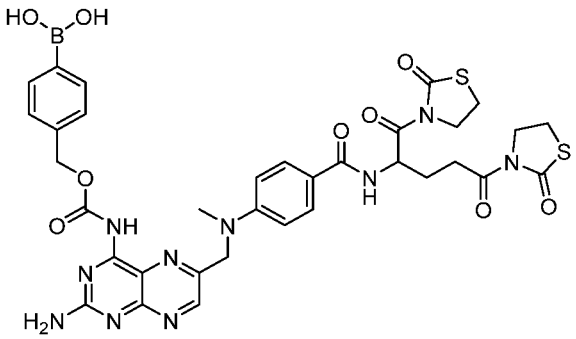
4-(4-(((2-Amino-4-((((4-boronobenzyl)oxy)carbonyl)amino)pteridin-6-yl)methyl)(methyl)amino)benzamido)-5-oxo-5-(2-oxothiazolidin-3-yl)pentanoic acid (45)



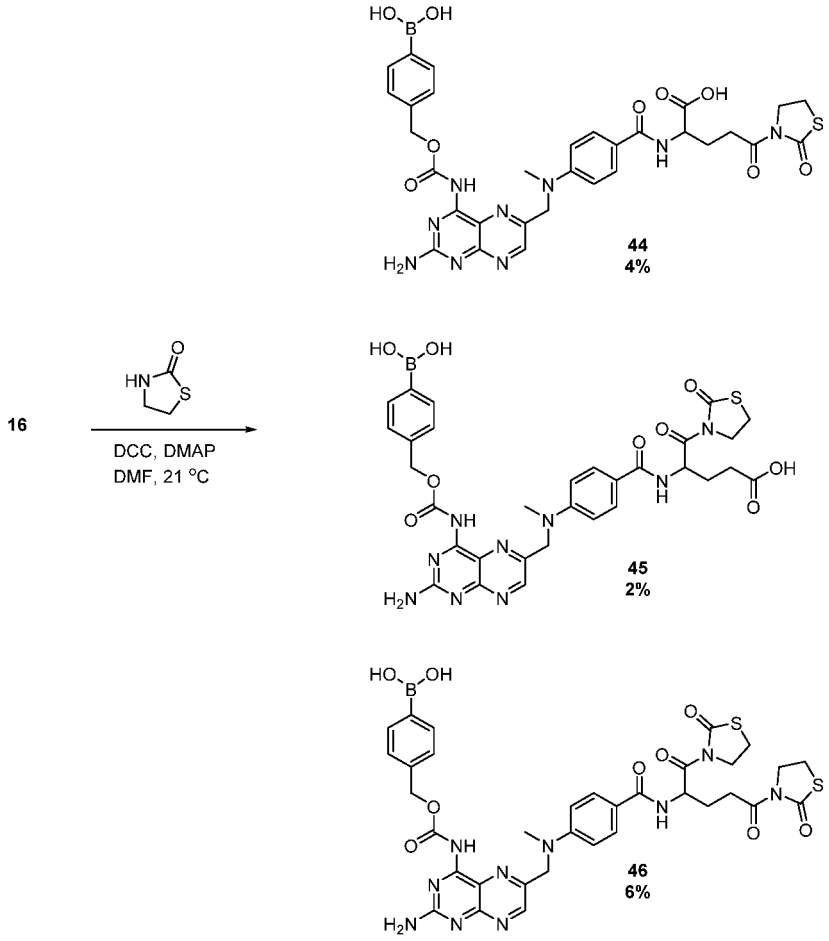
To a solution of **16** (100 mg, 0.16 mmol) in dry DMF (5 mL) was added DCC (55 mg, 0.27 mmol) followed by DMAP (58 mg, 0.47 mmol). The reaction mixture was stirred for 15 min under a N_2 atmosphere at 21 °C followed by addition of thiazolidin-2-one (28 mg, 0.27 mmol) and additional stirring for 16 h. Filtration of the crude reaction and concentration of the filtrate *in vacuo* afforded a residue which was purified by preparative HPLC to give the title compound **45** as a solid (2.0 mg, 2%). MS (ESI^+) m/z: calcd for $C_{31}H_{32}BN_9O_8S$ $[M+H-H_2O]^+$ 700.5, found 699.9; MS (ESI^-) m/z: calcd for $C_{31}H_{31}BN_9O_9S$ $[M-H]^-$ 716.2.5, found 716.3.

EXAMPLE 21

(4-((((2-Amino-6-(((4-((1,5-dioxo-1,5-bis(2-oxothiazolidin-3-yl)pentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)carbamoyl)oxy)methyl)phenyl)boronic acid (46)

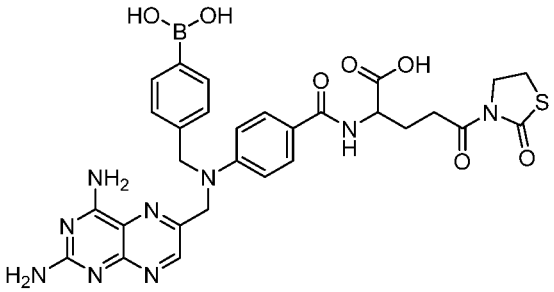


To a solution of **16** (100 mg, 0.16 mmol) in dry DMF (5 mL) was added DCC (82 mg, 0.39 mmol) followed by DMAP (77 mg, 0.63 mmol). The reaction mixture was stirred for 15 min under a N_2 atmosphere at 21 °C followed by addition of thiazolidin-2-one (41 mg, 0.39 mmol) and additional stirring for 16 h. Filtration of the crude reaction and concentration of the filtrate *in vacuo* afforded a residue which was purified by preparative HPLC to give the title compound **46** as a solid (8.0 mg, 6%). UPLC-MS (method C): R_t = 2.0 min; MS (ESI^+) m/z: calcd for $C_{34}H_{34}BN_{10}O_8S_2$ $[M+H-H_2O]^+$ 785.2, found 784.4.



EXAMPLE 22

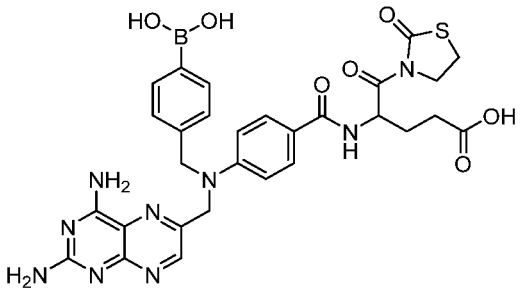
2-(4-((4-Boronobenzyl)((2,4-diaminopteridin-6-yl)methyl)amino)benzamido)-5-oxo-5-(2-oxothiazolidin-3-yl)pentanoic acid (47)



To a solution of **23** (95 mg, 0.16 mmol) in dry DMF (5 mL) was added DCC (55 mg, 0.27 mmol) followed by DMAP (58 mg, 0.47 mmol). The reaction mixture was stirred for 15 min under a N_2 atmosphere at 21 °C followed by addition of thiazolidin-2-one (28 mg, 0.27 mmol) and additional stirring for 16 h. Filtration of the crude reaction and concentration of the filtrate *in vacuo* afforded a residue which was purified by preparative HPLC to give the title compound **47** as a solid (4.0 mg, 4%). MS (ESI^+) m/z: calcd for $C_{29}H_{31}BN_9O_7S$ $[M+H]^+$ 660.2, found 660.2; MS (ESI^-) m/z: calcd for $C_{29}H_{29}BN_9O_7S$ $[M-H]^-$ 658.5, found 658.2.

EXAMPLE 23

4-(4-((4-Boronobenzyl)((2,4-diaminopteridin-6-yl)methyl)amino)benzamido)-5-oxo-5-(2-oxothiazolidin-3-yl)pentanoic acid (48)

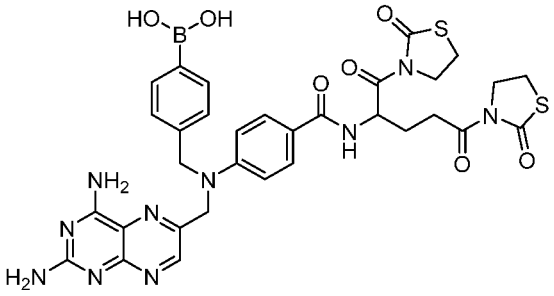


To a solution of **23** (95 mg, 0.16 mmol) in dry DMF (5 mL) was added DCC (55 mg, 0.27 mmol) followed by DMAP (58 mg, 0.47 mmol). The reaction mixture was stirred for 15 min under a N_2 atmosphere at 21 °C followed by addition of thiazolidin-2-one (28 mg, 0.27 mmol) and additional stirring for 16 h. Filtration of the crude reaction and concentration of the

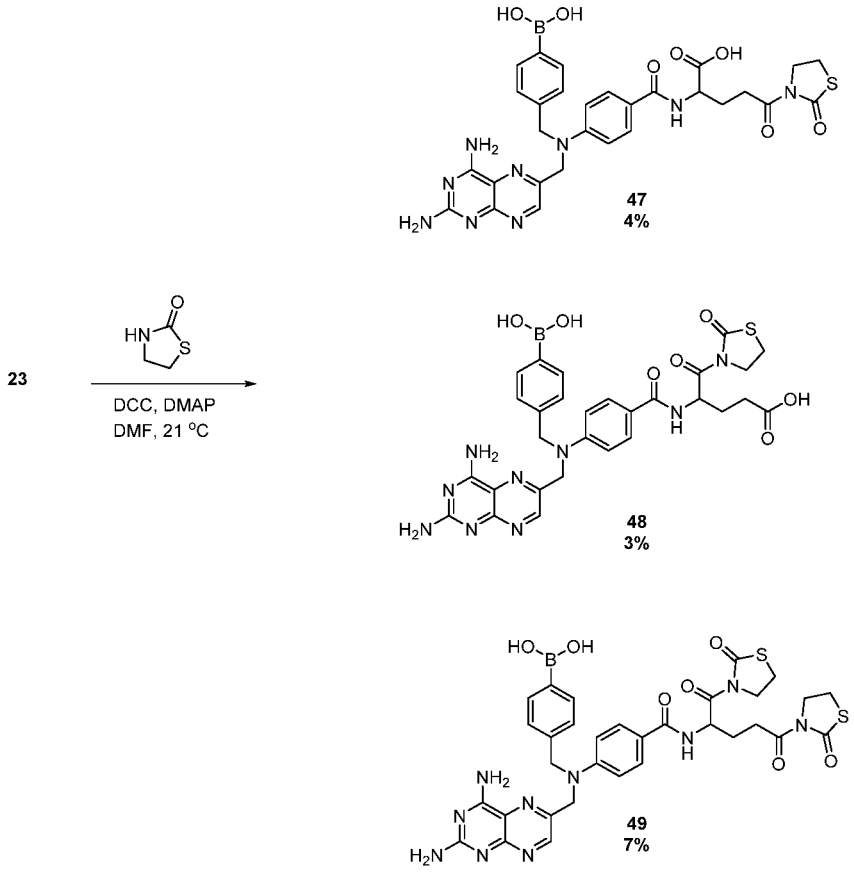
filtrate *in vacuo* afforded a residue which was purified by preparative HPLC to give the title compound **48** as a solid (3.1 mg, 3%). MS (ESI^+) m/z: calcd for $C_{32}H_{34}BN_{10}O_7S_2$ $[M+H]^+$ 660.2, found 660.2; MS (ESI^-) m/z: calcd for $C_{29}H_{29}BN_9O_7S$ $[M-H]^-$ 658.5, found 658.2

EXAMPLE 24

(4-((((2,4-Diaminopteridin-6-yl)methyl)(4-((1,5-dioxo-1,5-bis(2-oxothiazolidin-3-yl)pentan-2-yl)carbamoyl)phenyl)amino)methyl)phenyl)boronic acid (49)



To a solution of **23** (95 mg, 0.16 mmol) in dry DMF (5 mL) was added DCC (55 mg, 0.27 mmol) followed by DMAP (58 mg, 0.47 mmol). The reaction mixture was stirred for 15 min under a N_2 atmosphere at 21 °C followed by addition of thiazolidin-2-one (28 mg, 0.27 mmol) and additional stirring for 16 h. Filtration of the crude reaction and concentration of the filtrate *in vacuo* afforded a residue which was purified by preparative HPLC to give the title compound **49** as a solid (9.0 mg, 7%). UPLC-MS (method C): R_t = 1.95 min; MS (ESI^+) m/z: calcd for $C_{29}H_{31}BN_9O_7S$ $[M+H]^+$ 745.2, found 745.3.

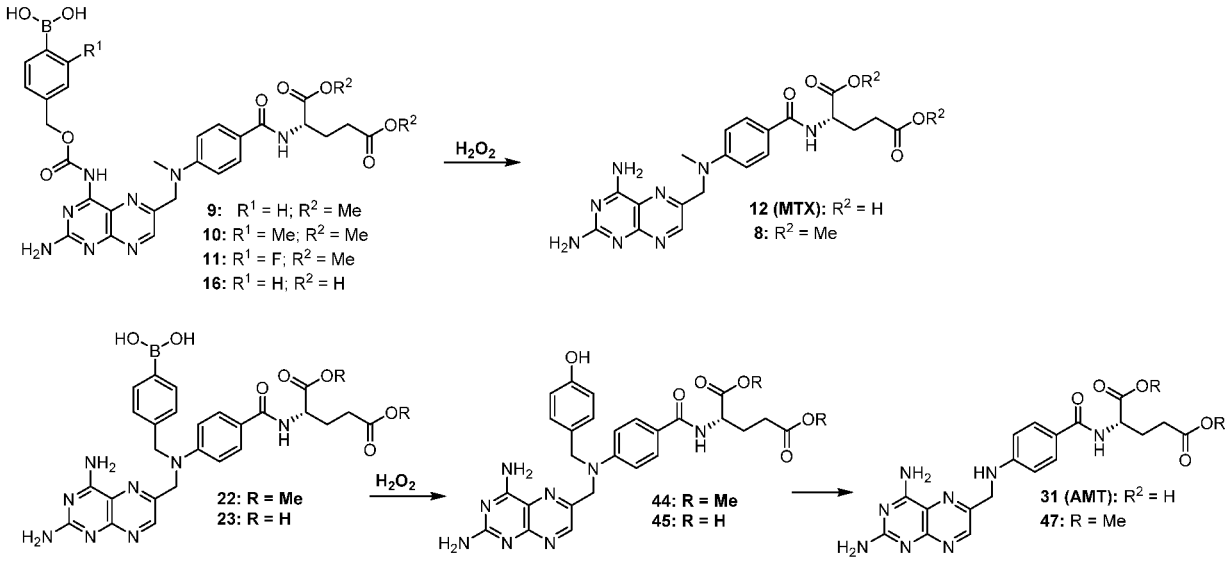


TEST EXAMPLE 1

***In vitro* assays**

Activation of prodrugs under oxidative conditions

To a mixture of DMSO (150 µL) and aq. PBS buffer (650 µL, pH 7.4) in an Eppendorf tube (1.5 mL) placed in a thermomixer at 37 °C was added a solution of prodrug (50 µL, 1 mM in DMSO) followed by addition of diclofenac as internal standard solution (50µL, 1 mM in DMSO). The assay was initiated by addition of a solution of H_2O_2 in PBS (50 µL, 10 mM) followed by vortex mixing. The resulting mixture was incubated at 37 °C at 1000 rpm and aliquots were analysed by RP-UPLC-MS after 5, 15, 30, 60, 90 min and 2, 3 and 4 h. Further data points were analysed for compounds **22** and **23** (16, 24 and 48 h). A control sample (no H_2O_2 addition but PBS) was run in parallel. Every prodrug activation assay was performed in triplicates.



Physicochemical and pharmacokinetic assays

Chemical Stability pH 7.4 / PBS stability. Chemical stability at pH 7.4 for compounds **16** and **23** was assessed using diclofenac as an internal standard. To 490 µL of a pre-warmed solution of 20 *µM* diclofenac in PBS (0.1% DMSO) is added 10 µL of a 1mM DMSO solution of compound **16** and **23** (in triplicates). The mixture was incubated at 1000 rpm at 37 °C in an Eppendorf Thermomixer C (1.5 mL) and samples were taken for analysis by RP-UPLC-MS after 30 min, 1h, 2h, 4h, 8h, 24h and 48h.

Chemical Stability (pH 7.4)

Prodrug	$t_{1/2}$ (h)
16	238
23	577

Solubility. Kinetic solubility at 100 µM with 1% DMSO**.** Briefly, the kinetic solubility, utilizing test compound from 10 mM DMSO stock solution, is measured at a final compound concentration of 100 µM and 1% DMSO. Test compound is added to 100 mM potassium

phosphate buffer, pH 7.4, and incubated at 37 °C for 20 hours in a heater-shaker. After incubation, the samples are centrifuged at 3000xg at 37 °C for 30 min to pellet insoluble material and an aliquot of the supernatant is taken for analysis. After dilution of the sample, the concentration of dissolved compound is quantified by LC-MS/MS analysis.

Plasma protein binding assay. Briefly, the fraction unbound drug (f_u) in plasma from human or other animal species was determined by equilibrium dialysis at 37 °C for 4 hours using a Rapid Equilibrium Dialysis (RED) device. The drug molecule at a concentration of 10 μM is added to 50% plasma and dialyzed against isotonic phosphate buffer (67 mM, pH 7.4). After dialysis, the drug concentration in the buffer and plasma is quantified by LC-MS/MS analysis. In parallel, the stability of the drug molecule in plasma is determined by incubating drug-spiked plasma (10 μM) at 37 °C for 4 hours, meanwhile the control plasma sample is kept in the freezer. The concentration of drug in both samples is quantified by LC-MS/MS analysis.

Pooled human plasma originating from healthy donors, 1 male and 1 female (non-smoking), was obtained from Uppsala Academic Hospital. Citrate was used as anticoagulant. CD1-1 Mouse plasma K2 EDTA is obtained from Innovative Research Inc. Plasma is stored frozen in aliquots to avoid repeated freeze-thaw cycles. 50% plasma is made by thawing human or animal plasma at rt. and mixing it with a equal volume of isotonic phosphate buffer.

Microsomal stability. The *in vitro* metabolic stability assay uses liver microsomes. Compound is dissolved in 100 mM KPO_4 buffer pH 7.4 to a 1μM final concentration. The assay is initiated by addition of NADPH and incubated for up to 40 min (THERMOstar, BMG Lab Technologies) with microsomes. Experiments are terminated at different time points by addition of acetonitrile. The amount of parent compound remaining is analyzed by LC-MS/MS. The natural logarithm of relative amount parent compound remaining is plotted against time and the first-order rate constant of consumption is determined by linear regression. *In vitro* half life is expressed in minutes and *in vitro* clearance in μl/(mg x min), respectively.

Cellular Assays

Cell Culture. The human breast cancer MCF-7 (Sigma) and human large cell lung cancer NCIH-460 (ATTC) cell lines were cultured in a humidified, 5% CO_2 atmosphere at 37 °C in Dulbecco´s Modified Eagle´s Medium (DMEM) (Sigma) or Roswell Park Memorial Institute medium (RPMI) 1640 (Sigma) supplemented with 10% fetal bovine serum (FBS, heat-inactivated, Fisher Scientific) and 1% penicillin/streptomycin. Both cell lines were subcultured every 2-3 days.

Pre-activation of compounds with H_2O_2. A 125 µM solution of tested compound was prepared in a 1.25 mM H_2O_2 in DMSO:PBS (1:1), placed in an Eppendorf tube and shaken at 21 °C for 24 h at 1000 rpm in an Eppendorf Thermomixer C (1.5 mL). The activation was followed by RP-UPLC-MS (λ = 306 nm) after 0 min, 15 min, 1 h, 4 h, and 24 h. A negative control consisting of a 125 µM solution of compound in a mixture of DMSO:PBS (1:1) without H_2O_2 was run in parallel to the activation assay in the same conditions.

Evaluation of cell viability. The 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-te-trazolium (MTS) assay (Promega Biotech AB, Stockholm, Sweden) was used to determine the in vitro antiproliferative effect of the compounds. This assay is based on the principle that cells have the ability to reduce MTS tetrazolium, while, when dead, they lose this ability. MCF-7 and NCI-H460 cells were cultured in 96-well plates at an initial density of 10^4 cells/well (MCF-7) or $7x10^3$ cells/well (NCI-H460) in their respective growth medium. After 24 h incubation to allow cell attachment, the medium was removed and the cells were incubated in the presence or absence of pre-activated compounds at different concentrations. After 48 h incubation time, the MTS reagent was added to each well. The cells were further incubated for a period of time between 30-60 min at 37 ºC until colorimetric reaction was developed within the linear range and the absorbance of the samples was measured at 490 nm using a 96-well plate spectrophotometer (Victor 3 plate reader with Wallac 1420 Workstation vs 3.0 software).

A control was used for each tested compound, where cells were incubated with DMEM or RPMI containing the equivalent concentration of DMSO (maximum of 0.4% v/v). Each concentration of tested compounds was done in triplicates. The final concentration of H2O2 in each well was always <10 µM (non-cytotoxic concentration in MCF-7 and NCI-H460 cell lines, determined with the described assay). The IC_{50} values were calculated using GraphPad Prism v6.0 (California, USA) as the concentration of the compounds required to cause 50% response compared to cells exposed to controls using a non-linear dose-response regression.

Table 1. Pharmacokinetic values of compound **16** and **23**.

Comp.	Kinetic solubility (μM)	Mouse Plasma Stability (%)[a,b]	Mouse $\%f_u$[b]	Mouse metabolic stability CL_{int} (mL/min/kg)[b,c]	Human plasma stability (%)[a, b]	Human $\%f_u$[b]	Human metabolic stability CL_{int} (mL/min/kg)[b,c]
16	41	72	64	177	88	0.26	2.94
23	83	>95	-	11	88	-	1.0

[a] **% compound recovered after 4 hours** [b] **pH = 7.4, 37 °C** [c] **using human microsomes**

Table 2. Evaluation of cell viability of compound **16**,methotrexate **12, 23,** and aminopterin **(31)** by MTS assay.[a]

	IC_{50} (nM)			
cell line	**MTX (12)**	**16**	**AMT (31)**	**23**
MCF-7	50 ± 24	44 ± 17	7.6 ± 3.4	2.6 ± 1.3
NCI-H460	12.3 ± 4.5	12.0 ± 4.4	7.7 ± 4.0	8.5 ± 4.1

[a] MCF-7 and NCI-H460 cells were incubated with tested compounds over 48 h, and their viability was determined using the MTS bioreduction assay.

FIGURE 1 shows RP-UPLC-MS UV (λ = 306 nm) chromatograms of the activation of **16** (t_R 0.93 min) at a concentration of 125 μM in a 1.25mM solution of H_2O_2 (10 eq) in DMSO:PBS (1:1). Data points were collected after 0 and 15 min, 1, 4 and 24h by RP-UPLC-MS. Methotrexate (**12**) elutes at t_R 0.62 min (identified with a commercially available reference sample). The peak at t_R 0.18 min corresponds to the solvent peak.

FIGURE 2 shows MCF-7 *in vitro* cell viability assay incubated with compounds **12** (methotrexate) and prodrug **16**. Cells were incubated at increasing concentrations of tested compouns for 48 h before MTS reagent was added. Results are calculated as mean of triplicates (mean ± SD, n = 3) and IC_{50} is calculated using a dose-response non-linear regression. Pre-activation of tested compounds with H_2O_2 was performed 24 h before experiment started as described in the experimental section.

FIGURE 3 shows *in vitro* cell viability study of MCF-7 cells incubated for 48 h with 0.25, 0.062 and 0.015 μM concentrations of compounds **12** (methotrexate) and **16** (mean ± SD, n = 3). Pre-activation of tested compounds with H_2O_2 was performed 24 h before experiment started as described in the experimental section.

FIGURE 4 shows NCI-H460 *in vitro* cell viability assay incubated with compounds **12** (methotrexate) and prodrug **16**. Cells were incubated at increasing concentrations of tested compouns for 48 h before MTS reagent was added. Results are calculated as mean of triplicates (mean ± SD, n = 3), and IC_{50} is calculated using a dose-response non-linear regression. Pre-activation of tested compounds with H_2O_2 was performed 24 h before experiment started as described in the experimental section.

FIGURE 5 shows *in vitro* cell viability study of NCI-H460 cells incubated for 48 h with 0.25, 0.062 and 0.015 μM concentrations of compounds **12** (methotrexate) and **16** (mean ± SD, n = 3). Pre-activation of tested compounds with H_2O_2 was performed 24 h before experiment started as described in the experimental section.

FIGURE 6 shows activation of prodrugs under oxidative conditions (H_2O_2). The activation was run at a test compound concentration of 50 μM and H_2O_2 of 0.5 mM in a mixture of 30% DMSO in PBS. The experiment was run in triplicates. Values are represented as mean and error bars as SD. Error bars not shown are smaller than the symbol.

FIGURE 7 shows NCI-H460 *in vitro* cell viability assay incubated with compounds **31** (aminopterin) and prodrug **23**. Cells were incubated at increasing concentrations of tested compounds for 48 h before MTS reagent was added. Results are calculated as mean of triplicates (mean ± SD, n = 3), and IC_{50} is calculated using a dose-response non-linear regression. Pre-activation of tested compounds with H_2O_2 was performed 24 h before experiment started as described in the experimental section.

FIGURE 8 shows MCF-7 *in vitro* cell viability assay incubated with compounds **31** (aminopterin) and prodrug **23**. Cells were incubated at increasing concentrations of tested compounds for 48 h before MTS reagent was added. Results are calculated as mean of triplicates (me an ± SD, n = 3) and IC_{50} is calculated using a dose-response non-linear regression. Pre-activation of tested compounds with H_2O_2 was performed 24 h before experiment started as described in the experimental section.

***In vivo* animal assay**

Materials and Methods

Animals: DBA/1J mice (male, 8-9 weeks) were obtained from Janvier, France. The mice were maintained in the animal house at Redoxis, Medicon Village, Lund, Sweden, where they were acclimatized for approximately one week before initiation of the experiment. All animal experiments were approved by the local animal ethic committee Malmö/Lund, Sweden, approved under the license N165-15.

*Induction of disease: collagen induced arthritis (*CIA) was induced by intradermal immunization with 100 µg of chicken type-II collagen (CII, Chondrex) in Complete Freund's Adjuvant (CFA, Difco) on day -1 *via* subcutaneous injection approximately 0.5 cm from the root of the tail. On day 21 a boost injection was administered in the same way with 50 µg CII. One week after the second immunization, onset of disease started to be observed (day 26).

Anti-arthritic effect of test compounds and health evaluation: mice were randomly divided in 5 groups (n = 8 per group): group I (vehicle), group II (**MTX**, Sigma Aldrich, 7.0 mg/kg, *i.p.*), group III (**AMT**, Enzo Life Sciences, 6.8 mg/kg, *i.p.*), group IV (**16**, 9.7 mg/kg, *i.p.*), group V (**23**, 8.8 mg/kg, *i.p.*). Vehicle and compound (2% DMSO in PBS, Life Technologies, injection volume 370 µL) were dosed daily intraperitoneally for 14 days, starting at onset of disease (day 27). Disease was evaluated three times per week in a blinded fashion, starting at day 18 until the end of the experiment (day 40). The reduction of swelling in the limbs was used as macroscopic score. A macroscopic scoring system of the four limbs ranging from 0 to 15 (1 point for each swollen or red toe) was used, meaning a maximal score of 60 per mice. For ethical reasons and restrictions, mice with score exceeding 45 were removed from the experiment. The general health of mice was evaluated three times per week after disease induction. As an indicator of general health, animal body weight was used.

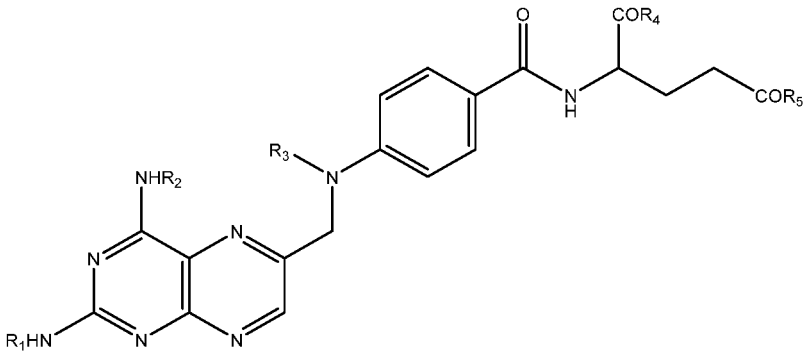
FIGURE 9 shows suppression of CIA development in mice after treatment with methotrexate (**MTX, 12**), aminopterin (**AMT, 31**) and prodrugs **16** and **23** (n = 8 per group). DBA/1J mice were given the indicated amounts of compound daily and disease progression vas evaluated three times per week starting on day 27. One animal in vehicle group and one animal in **23** group were sacrificed pre-termination due to high score. The AMT group was removed pre-termination due to a decline in health. Data represents mean values of arthritic score ± SEM. * represents a p-value <0.05 and ** represents a p-value <0.01 for comparison between MTX and vehicle, while † represents a p-value <0.05 for comparison between **16** and vehicle.

FIGURE 10 shows the general health of mice was evaluated three times per week during CIA as the average body weight in groups of animals (n = 8) tested with vehicle, **MTX, AMT, 16**, and **23**. One animal in vehicle group and one animal in **23** group were sacrificed pre-termination due to high score. The AMT group was removed pre-termination due to a decline in health. Data represents mean values of arthritic score ± SEM. * represents a p-value <0.05 for comparison between AMT and vehicle.

ASPECTS OF THE INVENTION

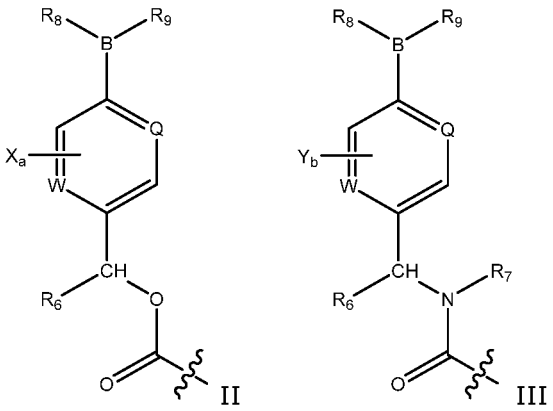
ASPECT 1

A compound having the formula I



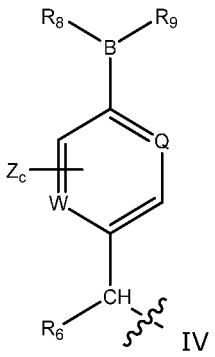
I

wherein R1 and R2 are selected from the group consisting of hydrogen and a moiety of the formula II, III or IV



II

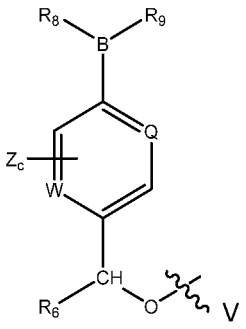
III



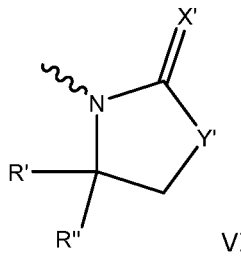
IV

R3 is selected from the group consisting of hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-4}alkynyl, C_{6-12}aryl, C_{4-11} heteroaryl and a moiety of the formula II, III or IV above,

R4 and R5 are independently selected from the group consisting of OH, O-C_{1-6}alkyl, O-C_{2-6}alkenyl, O-C_{6-12}aryl, O-C_{4-11}heteroaryl and a moiety of the formula V or VI;



V



VI

R6 and R7 are independently selected from the group consisting of hydrogen, CF_3, C_{1-6}alkyl, C_{2-6}alkenyl, C_{6-12}aryl, and C_{4-11}heteroaryl;

R8 and R9 are independently hydroxyl groups or R8 and R9 form, together with the intervening B and O atoms, a pinacol, catechol, diethanolamine, N-methyldiethanolamine or MIDA boronate group;

W and Q are independently C or N;

wherein each of X, Y and Z are selected from the group consisting of halogen, amino, nitro, cyano, hydroxyl, CF_3, C_{1-6}alkyl, C_{1-6}alkoxy, C_{2-6}alkenyl, C_{2-6}alkenyloxy, C_{6-12}aryl, C_{4-11}heteroaryl; wherein each of said C_{1-6}alkyl, C_{2-6}alkenyl, C_{6-12}aryl, C_{4-11}heteroaryl may be substituted by one or more substituents selected from the group consisting of halogen, amino, nitro, cyano, hydroxyl, CF_3, and C_{1-6}alkyl; and

each of a, b and c are integers in the range 0-4;

X' and Y' are independently S or O, and R' and R'' are independently selected from hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, aryl and C_{1-6}alkyl-aryl;

wherein, if each of R1, R2 and R3 are different from a moiety selected from a moiety of the formula II, III and IV, then at least one of R4 and R5 is a moiety of the formula V;

as well as pharmaceutically acceptable salts, solvates, and stereoisomers thereof.

ASPECT 2

The compound according to aspect 1, wherein W and Q are both C.

ASPECT 3

The compound according to aspect 1 or 2, wherein R6 and R7 are independently selected from the group consisting of hydrogen and C_{1-6}alkyl, preferably selected from the group consisting of hydrogen and C_{1-4}alkyl, preferably selected from the group consisting of hydrogen and methyl, preferably wherein R6 and R7 are both hydrogen.

ASPECT 4

The compound according to any one of the preceding aspects, wherein R8 and R9 are independently hydroxyl or R8 and R9 form, together with the intervening B and O atoms, a pinacol or catechol group, preferably wherein R8 and R9 are independently hydroxyl or R8 and R9 form, together with the intervening B and O atoms, a pinacol group, preferably wherein R8 and R9 are both hydroxyl groups.

ASPECT 5

The compound according to any one of the preceding aspects, wherein each of X, Y and Z are selected from the group consisting of halogen, cyano, hydroxyl, CF_3, and C_{1-6}alkyl; and each of a, b and c are 0, 1 or 2.

ASPECT 6

The compound according to any one of the preceding aspects, wherein each of X, Y and Z are selected from the group consisting of halogen, cyano, hydroxyl, and C_{1-6}alkyl; preferably wherein each of X, Y and Z are selected from the group consisting of halogen and C_{1-4}alkyl; preferably wherein each of X, Y and Z are selected from the group consisting of fluoro and methyl; and each of a, b and c are 0 or 1.

ASPECT 7

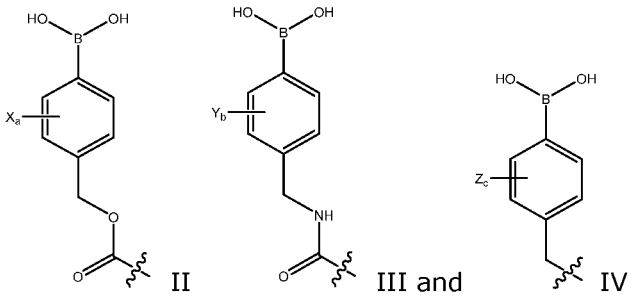
The compound according to any one of the preceding aspects, wherein Y' is S.

ASPECT 8

The compound according to any one of the preceding aspects, wherein X' is O.

ASPECT 9

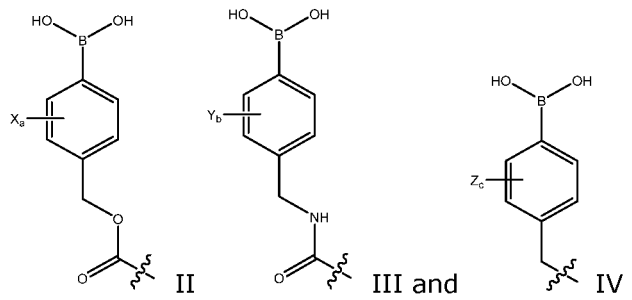
The compound according to any one of the preceding aspects, wherein R2 is selected from the group consisting of



in which X, Y, Z, a, b and c are as defined in any one of aspects 1-8;
R1 is hydrogen;
R3 is selected from the group consisting of hydrogen and C_{1-6}alkyl, preferably selected from the group consisting of hydrogen and C_{1-4}alkyl, preferably wherein said R3 is methyl; and
R4 and R5 are selected from the group consisting of OH and O-C_{1-6}alkyl, preferably selected from the group consisting of OH and O-C_{1-4}alkyl, preferably wherein R4 and R5 are both methoxy or hydroxy.

ASPECT 10

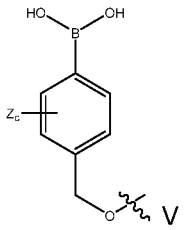
The compound according to any one of aspects 1-8, wherein R3 is selected from the group consisting of



in which X, Y, Z, a, b and c are as defined in any one of aspects 1-8;
R1 and R2 are hydrogen; and
R4 and R5 are selected from the group consisting of OH and O-C_{1-6}alkyl, preferably selected from the group consisting of OH and O-C_{1-4}alkyl, preferably wherein R4 and R5 are both methoxy or hydroxy.

ASPECT 11

The compound according to any one of aspects 1-8, wherein R4 and/or R5 is a moiety of the formula



in which Z and c are as defined in any one of aspects 1-8;
R1 and R2 are hydrogen; and
R3 is selected from the group consisting of hydrogen and C_{1-6}alkyl, preferably selected from the group consisting of hydrogen and C_{1-4}alkyl, preferably wherein said R3 is methyl.

ASPECT 12

The compound according to any one of the preceding aspects selected from the group consisting of

(*S*)-(4-((((2-amino-6-(((4-((1,5-dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)carbamoyl)oxy)methyl)phenyl)boronic acid (compound **9**)

(*S*)-(4-((((2-amino-6-(((4-((1,5-dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)carbamoyl)oxy)methyl)-2-methylphenyl)boronic acid (compound **10**)

(*S*)-(4-((((2-amino-6-(((4-((1,5-dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)carbamoyl)oxy)methyl)-2-fluorophenyl)boronic acid (compound **11**)

Bis(4-methoxybenzyl) (4-(((2-amino-4-((((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)carbonyl)amino)pteridin-6-yl)methyl)(methyl)amino)benzoyl)-L-glutamate (compound **14**)

(*S*)-(4-((((2-amino-6-(((4-((1,5-bis((4-methoxybenzyl)oxy)-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)carbamoyl)oxy)methyl)phenyl)boronic acid (compound **15**)

(4-(((2-amino-4-((((4-boronobenzyl)oxy)carbonyl)amino)pteridin-6-yl)methyl)(methyl)amino)benzoyl)-L-glutamic acid (compound **16**)

(*S*)-(4-((((2,4-diaminopteridin-6-yl)methyl)(4-((1,5-dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)amino)methyl)phenyl)boronic acid (compound **22**)

(4-((4-boronobenzyl)((2,4-diaminopteridin-6-yl)methyl)amino)benzoyl)-L-glutamic acid (compound **23**),

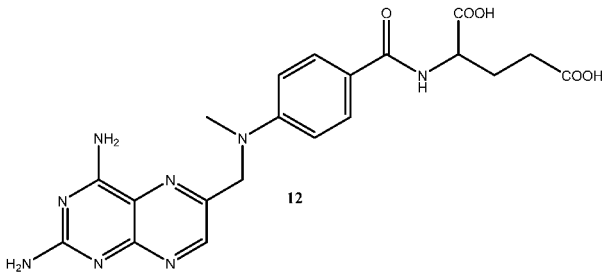
(*S*)-(4-((((2-amino-6-(((4-((1,5-di-*tert*-butoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)carbamoyl)oxy)methyl)phenyl)boronic acid (compound **30**)

(4-((((2-amino-6-(((4-((1,5-bis(allyloxy)-1,5-dioxopentan-2 yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)carbamoyl)oxy)methyl)phenyl)boronic acid (compound **33**).

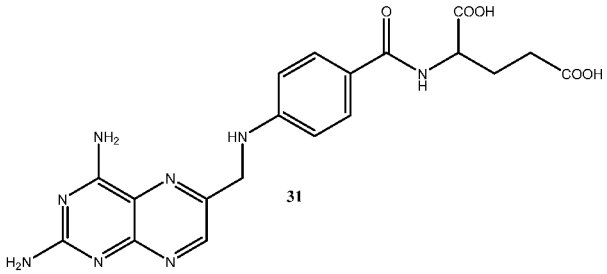
ASPECT 13

A method for the preparation of a compound of the formula I, comprising the steps:

a) Providing methotrexate (MTX) of the formula **12** or aminopterin of the formula **31** or any protected versions of them

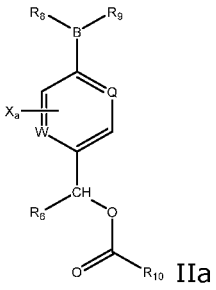


12

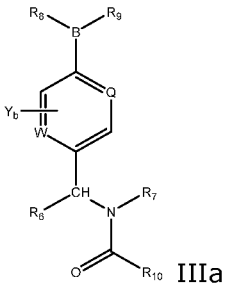


31

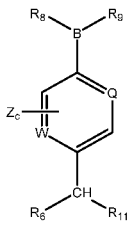
b) Providing a compound of formula IIa, IIIa, or Iva



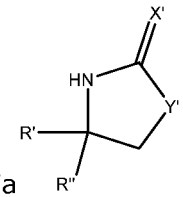
IIa



IIIa



IVa



VI

Wherein R4, R5, R6, R7, R8, R9, R', R'', W, Q, X, X', Y, Y' Z, a, b, c are as defined above,

R10 and R11 is a leaving group LG; and

c) Reacting optionally protected MTX(**12**) or optionally protected aminopterin (**31**) with a compound of formula IIa, IIIa, IVa, or VI to obtain a compound of formula I according to the invention;

d) Optionally performing a deprotection step.

ASPECT 14

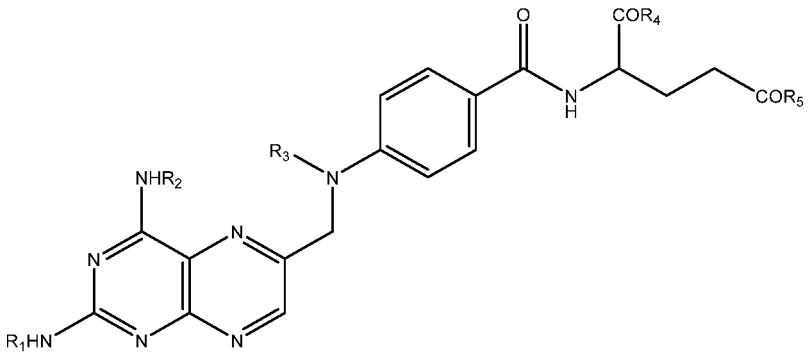
A pharmaceutical composition comprising a compound according to any one of aspects 1-12 optionally in combination with one or more excipients.

ASPECT 15

A compound according to any one of aspects 1-12 as a prodrug for the treatment of inflammatory diseases or cancer, such as wherein said inflammatory disease is selected from the group consisting of rheumatoid arthritis (RA), juvenile dermatomyositis, psoriasis, psoriatic arthritis, lupus, sarcoidosis, atopic dermatitis, eczema Crohn's disease, colitis ulcerosa, multiple sclerosis, and Amyotropic Lateral Sclerosis (ALS).

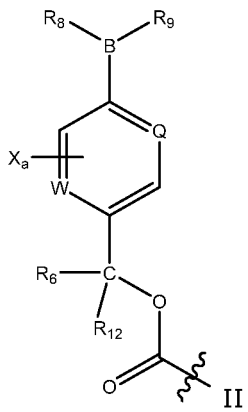
CLAIMS

1. A compound having the formula I

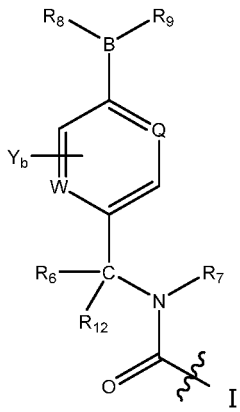


I

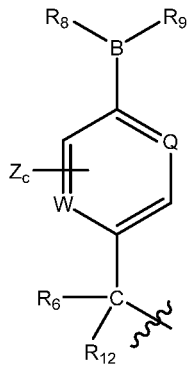
wherein R1 and R2 are independently selected from the group consisting of hydrogen and a moiety of the formula II, III, IV, VII, VIII, IX, X, XI, or XII



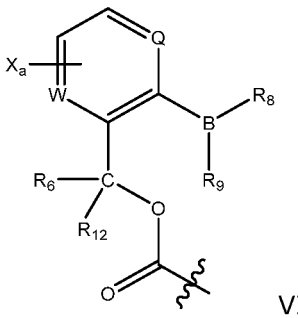
II



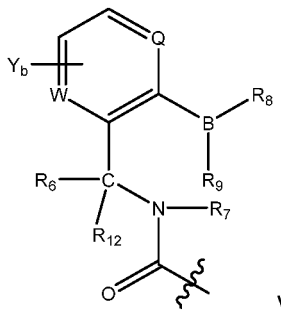
III



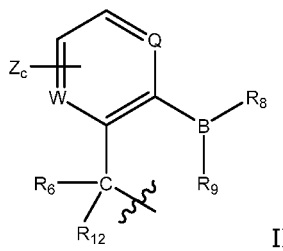
IV



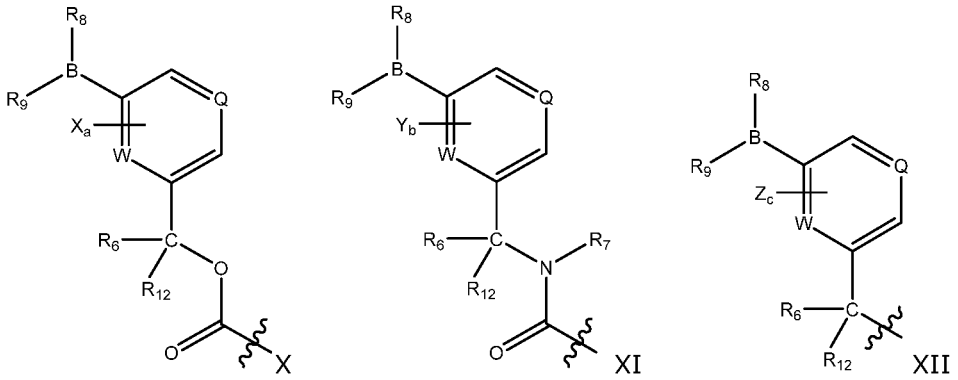
VII



VIII

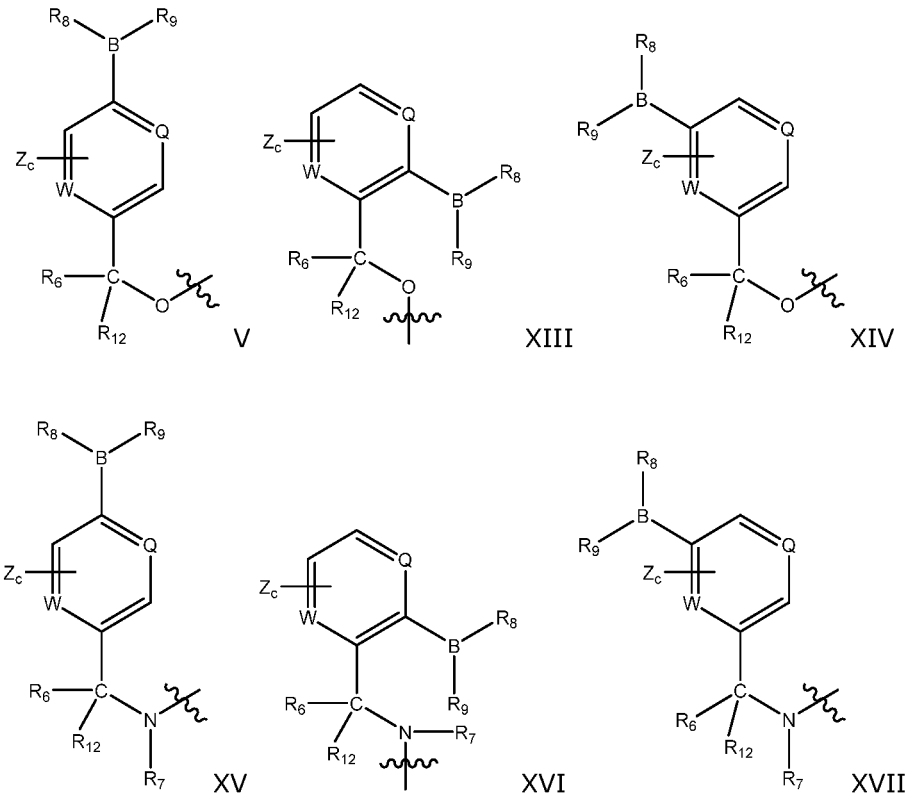


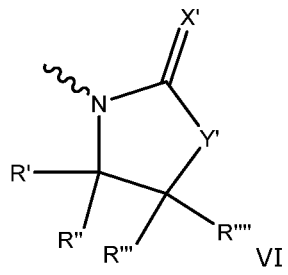
IX



R3 is selected from the group consisting of hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-4}alkynyl, C_{6-12}aryl, C_{4-11} heteroaryl and a moiety of the formula II, III, IV, VII, VIII, IX, X, XI, or XII above;

R4 and R5 are independently selected from the group consisting of OH, O-C_{1-6}alkyl, O-C_{2-6}alkenyl, O-C_{6-12}aryl, O-C_{4-11}heteroaryl and a moiety of the formula V, VI, XIII, XIV, XV, XVI, or XVII;





VI

R6, R7 and R12 are independently selected from the group consisting of hydrogen, CF_3, C_{1-6}alkyl, C_{2-6}alkenyl, C_{6-12}aryl, and C_{4-11}heteroaryl;

R8 and R9 are independently hydroxyl groups or R8 and R9 form, together with the intervening B and O atoms, a pinacol, catechol, diethanolamine, N-methyldiethanolamine or N-methyliminodiacetic acid (MIDA) boronate group;

W and Q are independently C or N;

wherein each of X, Y and Z are selected from the group consisting of halogen, amino, nitro, cyano, hydroxyl, CF_3, C_{1-6}alkyl, C_{1-6}alkoxy, C_{2-6}alkenyl, C_{2-6}alkenyloxy, C_{6-12}aryl, C_{4-11}heteroaryl; wherein each of said C_{1-6}alkyl, C_{2-6}alkenyl, C_{6-12}aryl, C_{4-11}heteroaryl may be substituted by one or more substituents selected from the group consisting of halogen, amino, nitro, cyano, hydroxyl, CF_3, and C_{1-6}alkyl; and

each of a, b and c are integers in the range 0-4;

X' and Y' are independently S or O, and R', R'', R''' and R'''' are independently selected from hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{6-12}aryl, C_{4-11}heteroaryl, and C_{1-6}alkyl-C_{6-12}aryl;

wherein, if each of R1, R2 and R3 are different from a moiety selected from a moiety of the formula II, III, IV, VII, VIII, IX, X, XI, or XII then at least one of R4 and R5 is a moiety of the formula V, XIII, XIV, XV, XVI, or XVII;

as well as pharmaceutically acceptable salts, solvates, and stereoisomers thereof.

2. The compound according to claim 1, wherein R1 and R2 are independently selected from the group consisting of hydrogen and a moiety of the formula II, III, or IV.

3. The compound according to claim 1 or 2, wherein R3 is selected from the group consisting of hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-4}alkynyl, C_{6-12}aryl, C_{4-11}heteroaryl and a moiety of the formula II, III, or IV.

4. The compound according to any one of the preceding claims, wherein R4 and R5 are independently selected from the group consisting of OH, O-C_{1-6}alkyl, O-C_{2-6}alkenyl, O-C_{6-12}aryl, O-C_{4-11}heteroaryl and a moiety of the formula V, or VI.

5. The compound according to any one of the preceding claims, wherein R1 and R2 are independently selected from the group consisting of hydrogen and a moiety of the formula II, III, or IV;

R3 is selected from the group consisting of hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-4}alkynyl, C_{6-12}aryl, C_{4-11} heteroaryl and a moiety of the formula II, III, or IV;

R4 and R5 are independently selected from the group consisting of OH, O-C_{1-6}alkyl, O-C_{2-6}alkenyl, O-C_{6-12}aryl, O-C_{4-11}heteroaryl and a moiety of the formula V, or VI; and

wherein, if each of R1, R2 and R3 are different from a moiety selected from a moiety of the formula II, III, and IV, then at least one of R4 and R5 is a moiety of the formula V.

6. The compound according to claim 1, wherein W and Q are both C.

7. The compound according to any one of the preceding claims, wherein R6 and R7 are independently selected from the group consisting of hydrogen and C_{1-6}alkyl, preferably selected from the group consisting of hydrogen and C_{1-4}alkyl, preferably selected from the group consisting of hydrogen and methyl, preferably wherein R6 and R7 are both hydrogen.

8. The compound according to any one of the preceding claims, wherein R8 and R9 are independently hydroxyl or R8 and R9 form, together with the intervening B and O atoms, a pinacol or catechol group, preferably wherein R8 and R9 are independently hydroxyl or R8 and R9 form, together with the intervening B and O atoms, a pinacol group, preferably wherein R8 and R9 are both hydroxyl groups.

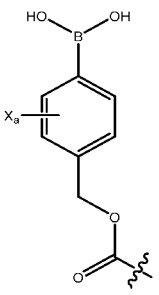
9. The compound according to any one of the preceding claims, wherein each of X, Y and Z are selected from the group consisting of halogen, cyano, hydroxyl, CF_3, and C_{1-6}alkyl; and each of a, b and c are 0, 1 or 2.

10. The compound according to any one of the preceding claims, wherein each of X, Y and Z are selected from the group consisting of halogen, cyano, hydroxyl, and C_{1-6}alkyl; preferably wherein each of X, Y and Z are selected from the group consisting of halogen and C_{1-4}alkyl; preferably wherein each of X, Y and Z are selected from the group consisting of fluoro and methyl; and each of a, b and c are 0 or 1.

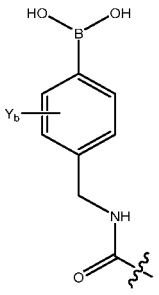
11. The compound according to any one of the preceding claims, wherein Y' is S.

12. The compound according to any one of the preceding claims, wherein X' is O.

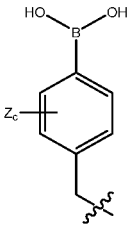
13. The compound according to any one of the preceding claims, wherein R2 is selected from the group consisting of



II



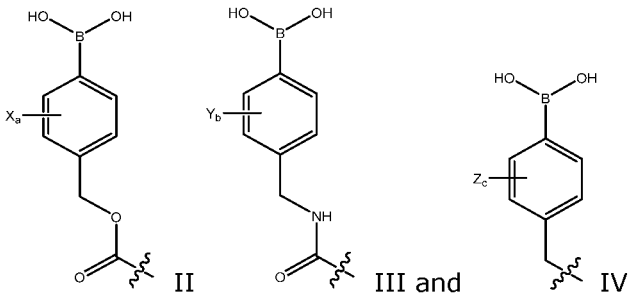
III and



IV

in which X, Y, Z, a, b and c are as defined in any one of claims 1-12;
R1 is hydrogen;
R3 is selected from the group consisting of hydrogen and C_{1-6}alkyl, preferably selected from the group consisting of hydrogen and C_{1-4}alkyl, preferably wherein said R3 is methyl; and
R4 and R5 are selected from the group consisting of OH and O-C_{1-6}alkyl, preferably selected from the group consisting of OH and O-C_{1-4}alkyl, preferably wherein R4 and R5 are both methoxy or hydroxy.

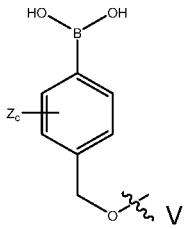
14. The compound according to any one of claims 1-12, wherein R3 is selected from the group consisting of



II III and IV

in which X, Y, Z, a, b and c are as defined in any one of claims 1-12;
R1 and R2 are hydrogen; and
R4 and R5 are selected from the group consisting of OH and O-C_{1-6}alkyl, preferably selected from the group consisting of OH and O-C_{1-4}alkyl, preferably wherein R4 and R5 are both methoxy or hydroxy.

15. The compound according to any one of claims 1-12, wherein R4 and/or R5 is a moiety of the formula



V

in which Z and c are as defined in any one of claims 1-12;
R1 and R2 are hydrogen; and
R3 is selected from the group consisting of hydrogen and C_{1-6}alkyl, preferably selected from the group consisting of hydrogen and C_{1-4}alkyl, preferably wherein said R3 is methyl.

16. The compound according to any one of the preceding claims selected from the group consisting of

(*S*)-(4-((((2-Amino-6-(((4-((1,5-dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)carbamoyl)oxy)methyl)phenyl)boronic acid (compound **9**)

(*S*)-(4-((((2-Amino-6-(((4-((1,5-dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)carbamoyl)oxy)methyl)-2-methylphenyl)boronic acid (compound **10**)

(*S*)-(4-((((2-Amino-6-(((4-((1,5-dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)carbamoyl)oxy)methyl)-2-fluorophenyl)boronic acid (compound **11**)

Bis(4-methoxybenzyl) (4-(((2-amino-4-((((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)carbonyl)amino)pteridin-6-yl)methyl)(methyl)amino)benzoyl)-*L*-glutamate (compound **14**)

(*S*)-(4-((((2-Amino-6-(((4-((1,5-bis((4-methoxybenzyl)oxy)-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)carbamoyl)oxy)methyl)phenyl)boronic acid (compound **15**)

(4-(((2-Amino-4-((((4-boronobenzyl)oxy)carbonyl)amino)pteridin-6-yl)methyl)(methyl)amino)benzoyl)-*L*-glutamic acid (compound **16**)

(*S*)-(4-((((2,4-Siaminopteridin-6-yl)methyl)(4-((1,5-dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)amino)methyl)phenyl)boronic acid (compound **22**)

(4-((4-Boronobenzyl)((2,4-diaminopteridin-6-yl)methyl)amino)benzoyl)-*L*-glutamic acid (compound **23**),

(*S*)-(4-((((2-Amino-6-(((4-((1,5-di-*tert*-butoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)carbamoyl)oxy)methyl)phenyl)boronic acid (compound **30**)

(4-((((2-Amino-6-(((4-((1,5-bis(allyloxy)-1,5-dioxopentan-2 yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)carbamoyl)oxy)methyl)phenyl)boronic acid (compound **33**)

(*S*)-(4-((3-(2-Amino-6-(((4-((1,5-dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)ureido)methyl)phenyl)boronic acid **(**compound **35)**

(S)-5-((4-Boronobenzyl)oxy)-4-(4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)benzamido)-5-oxopentanoic acid **(**compound **37)**

(S)-5-((4-Boronobenzyl)oxy)-2-(4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)benzamido)-5-oxopentanoic acid **(**compound **38)**

(*S*)-(4-(((5-((4-Boronobenzyl)oxy)-2-(4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)benzamido)-5-oxopentanoyl)oxy)methyl)phenyl)boronic acid (compound **39)**

(*S*)-(4-(((2-Amino-6-(((4-((1,5-dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)amino)methyl)phenyl)boronic acid (compound **40)**

(*S*)-(4-(((4-Amino-6-(((4-((1,5-dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)-phenyl)(methyl)amino)methyl)pteridin-2-yl)amino)methyl)phenyl)boronic acid (compound **41)**

(*S*)-((((6-(((4-((1,5-Dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)-(methyl)amino)methyl)pteridine-2,4-diyl)bis(azanediyl))bis(methylene))bis(4,1-phenylene))diboronic acid (compound **42)**

(*S*)-((((((6-(((4-((1,5-Dimethoxy-1,5-dioxopentan-2-yl)carbamoyl)phenyl)-(methyl)amino)methyl)pteridine-2,4-diyl)bis(azanediyl))bis(carbonyl))bis(oxy))-bis(methylene))bis(4,1-phenylene))diboronic acid (compound **43)**

(2-(4-(((2-Amino-4-((((4-boronobenzyl)oxy)carbonyl)amino)pteridin-6-yl)methyl)(methyl)amino)benzamido)-5-oxo-5-(2-oxothiazolidin-3-yl)pentanoic acid (compound **44)**

4-(4-(((2-Amino-4-((((4-boronobenzyl)oxy)carbonyl)amino)pteridin-6-yl)methyl)(methyl)amino)benzamido)-5-oxo-5-(2-oxothiazolidin-3-yl)pentanoic acid (compound **45)**

(4-((((2-Amino-6-(((4-((1,5-dioxo-1,5-bis(2-oxothiazolidin-3-yl)pentan-2-yl)carbamoyl)phenyl)(methyl)amino)methyl)pteridin-4-yl)carbamoyl)oxy)-methyl)phenyl)boronic acid (compound **46)**

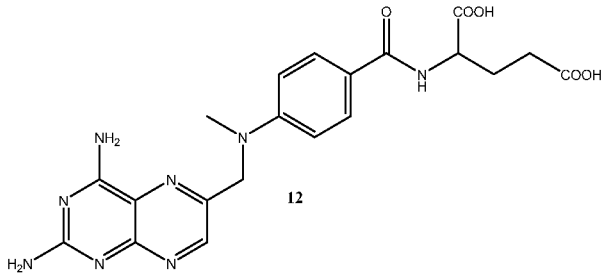
2-(4-((4-Boronobenzyl)((2,4-diaminopteridin-6-yl)methyl)amino)benzamido)-5-oxo-5-(2-oxothiazolidin-3-yl)pentanoic acid (compound **47)**

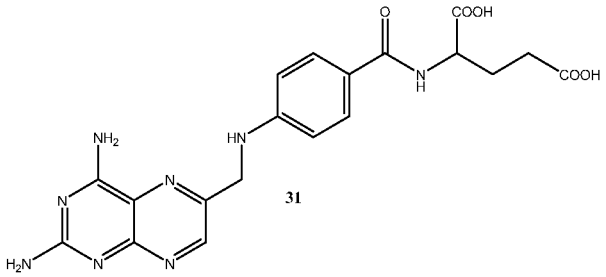
4-(4-((4-Boronobenzyl)((2,4-diaminopteridin-6-yl)methyl)amino)benzamido)-5-oxo-5-(2-oxothiazolidin-3-yl)pentanoic acid (compound **48)**

(4-((((2,4-Diaminopteridin-6-yl)methyl)(4-((1,5-dioxo-1,5-bis(2-oxothiazolidin-3-yl)pentan-2-yl)carbamoyl)phenyl)amino)methyl)phenyl)boronic acid (compound **49**).

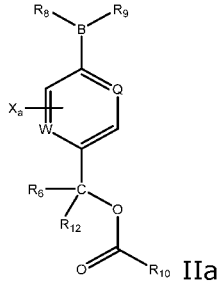
17. A method for the preparation of a compound of the formula I, comprising the steps:

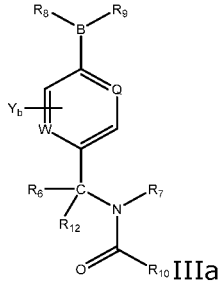
a) Providing methotrexate (MTX) of the formula **12** or aminopterin of the formula **31** or any protected versions of them





b) Providing a compound of formula IIa, IIIa, IVa, VIa, VIIa, VIIIa, IXa, Xa, XIa, XIIa, XVIIIa, XIXa, XXa, XXIa, XXXIIa, or XXIIIa


IIa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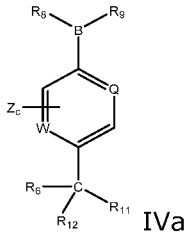
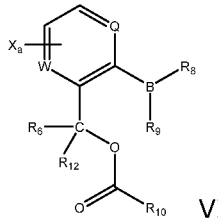
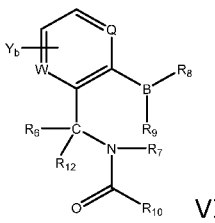

IVa


VIIa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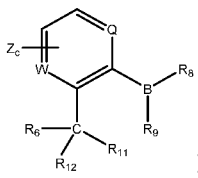
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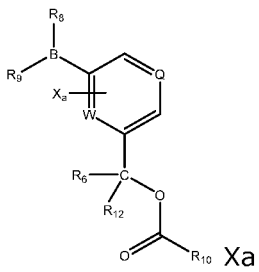
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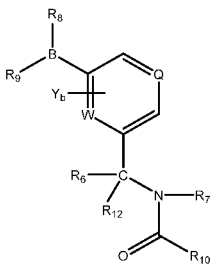
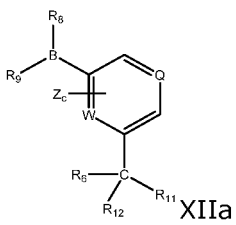

XIa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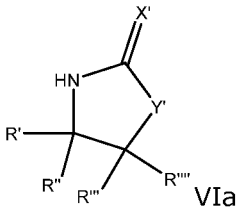
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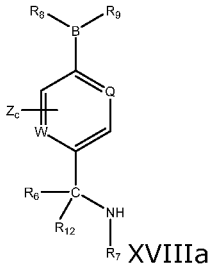
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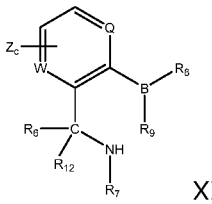
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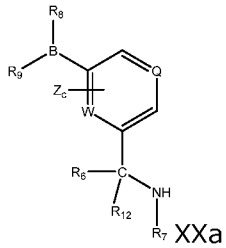
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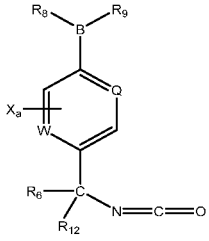
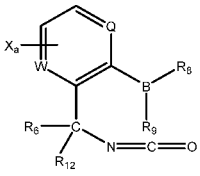
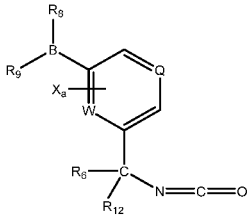

XXIa


XXIIa


XXIIIa

Wherein R4, R5, R6, R7, R8, R9, R12, R', R'', R''', R'''', W, Q, X, X', Y, Y' Z, a, b, c are as defined above,

R10 and R11 is a leaving group LG; and

c) Reacting optionally protected MTX (**12**) or optionally protected aminopterin (**31**) with a compound of formula IIa, IIIa, IVa, VIIa, VIIIa, IXa, Xa, XIa, XIIa, XVIIIa, XIXa, XXa, XXIa, XXIIa, or XXIIIA to obtain a compound of formula I according to the invention;

d) Optionally performing a deprotection step;

e) Optionally reacting the compound obtained in step c) or d), as appropriate, with a compound of the formula IIa, IIIa, IVa, VIa, VIIa, VIIIa, IXa, Xa, XIa, XIIa, XVIIIa, XIXa, XXa, XXIa, XXIIa, or XXIIIa, followed by an optional deprotection step to obtain a compound of formula I according to the invention.

18. A pharmaceutical composition comprising a compound according to any one of claims 1-16 optionally in combination with one or more excipients.

19. A compound according to any one of claims 1-16 for the treatment of inflammatory diseases or cancer.

20. The compound according to claim 19, wherein said inflammatory diseases are selected from the group consisting of rheumatoid arthritis (RA), juvenile dermatomyositis, juvenile rheumatoid arthritis, psoriasis, psoriatic arthritis, lupus, sarcoidosis, atopic dermatitis, eczema, Crohn's disease, uveitis associated with juvenile idiopathic arthritis or ulcerative

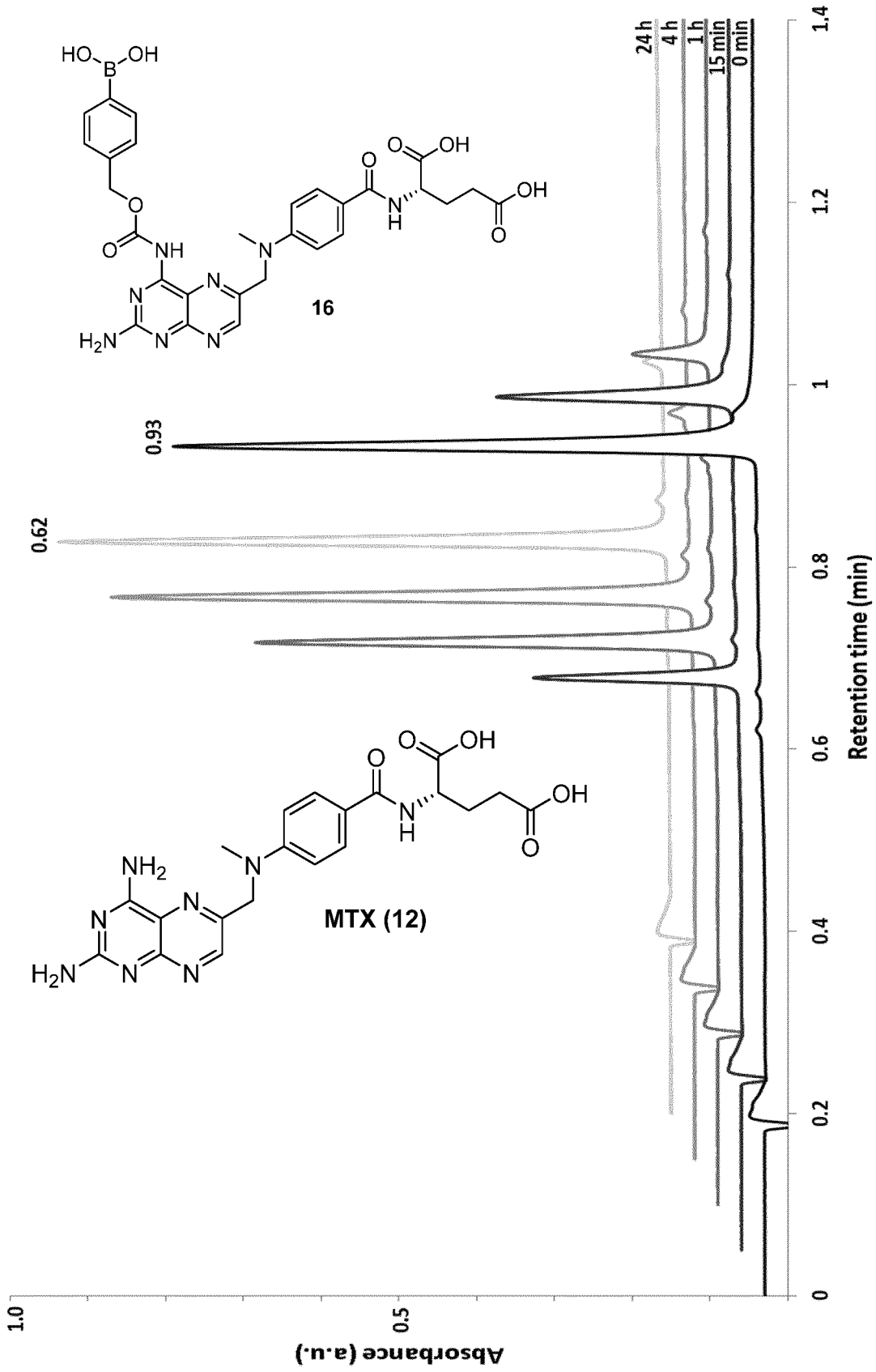
colitis, colitis ulcerosa, multiple sclerosis, Amyotropic Lateral Sclerosis (ALS), non-infectious ocular inflammation, vasculitis, systemic lupus erythematosus, and eosinophilic fasciitis.

21. The compound according to claim 19, wherein said cancer is selected from the group consisting of acute lymphocytic leukemia, meningeal leukemia, myeloproliferative neoplasm, breast cancer, squamous cell carcinoma, lymphosarcoma, osteosarcoma, advanced mycosis fungoides (cutaneous T cell lymphoma), small cell types lung cancer, non-small cell lung cancer, and non-Hodgkin's lymphoma.

22. A method for the treatment of a patient suffering from inflammatory diseases or cancer, said method comprising administering a compound according to any one of claims 1-16 as a prodrug for the treatment of inflammatory diseases or cancer.

23. The method according to claim 22, wherein said inflammatory disease is selected from the group consisting of rheumatoid arthritis (RA), juvenile dermatomyositis, juvenile rheumatoid arthritis, psoriasis, psoriatic arthritis, lupus, sarcoidosis, atopic dermatitis, eczema, Crohn's disease, uveitis associated with juvenile idiopathic arthritis or ulcerative colitis, colitis ulcerosa, multiple sclerosis, Amyotropic Lateral Sclerosis (ALS), non-infectious ocular inflammation, vasculitis, systemic lupus erythematosus, and eosinophilic fasciitis.

24. The method according to claim 22, wherein said cancer is selected from the group consisting of acute lymphocytic leukemia, meningeal leukemia, myeloproliferative neoplasm, breast cancer, squamous cell carcinoma, lymphosarcoma, osteosarcoma, advanced mycosis fungoides (cutaneous T cell lymphoma), small cell types lung cancer, non-small cell lung cancer, and non-Hodgkin's lymphoma.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0.93
0.62
16
MTX (12)
Retention time (min)
Absorbance (a.u.)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0.5
1.0

FIG. 1

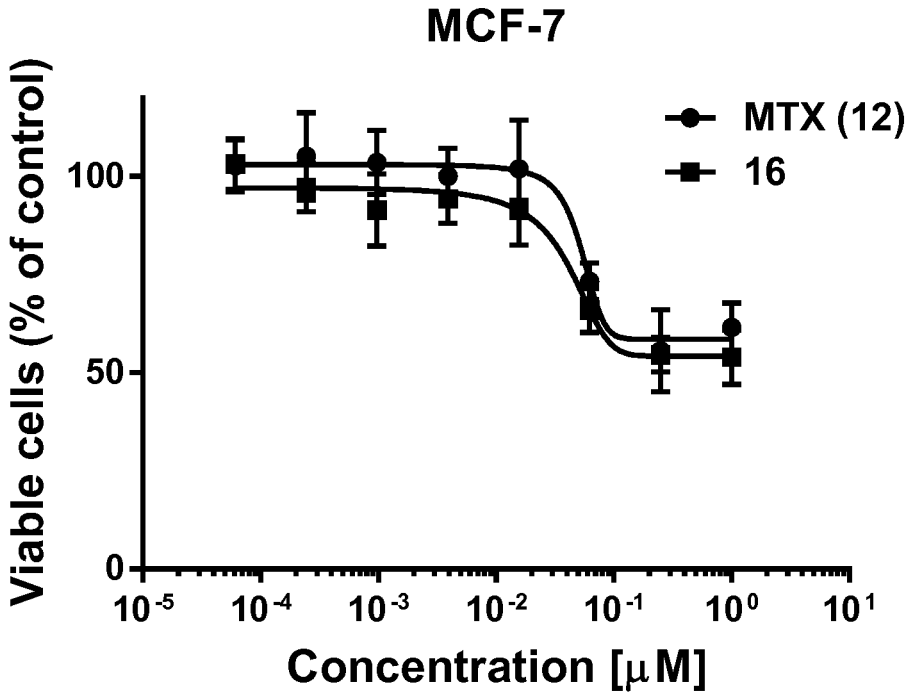
MCF-7
MTX (12)
16
Viable cells (% of control)
100
50
0
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
Concentration [μM]

FIG. 2

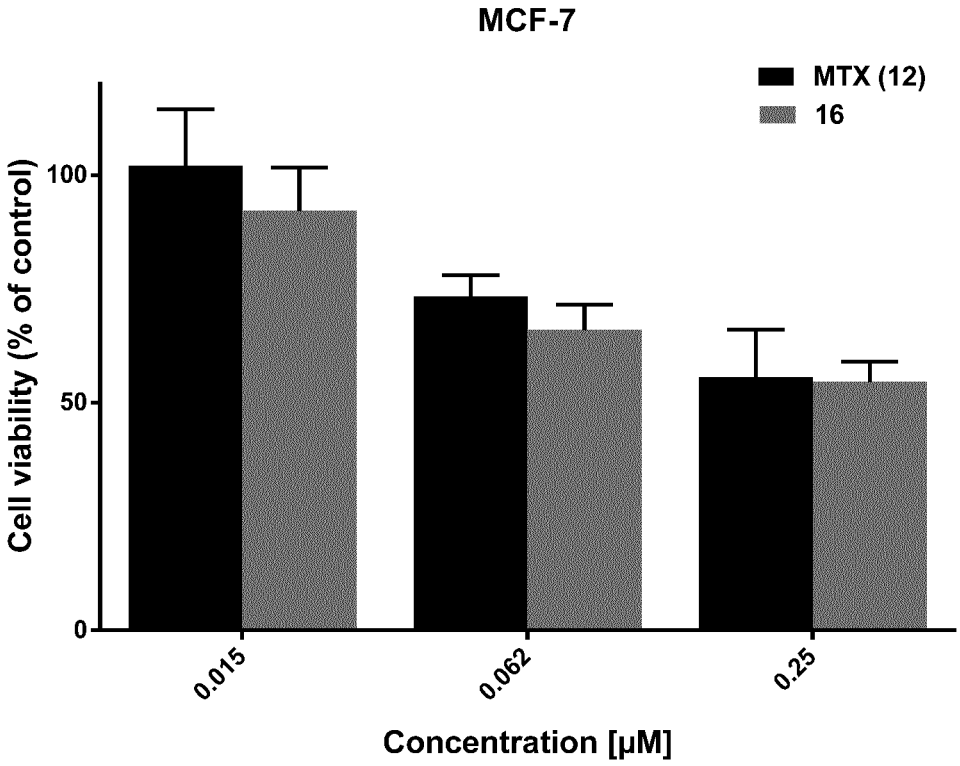
MCF-7
MTX (12)
16
Cell viability (% of control)
100
50
0
0.015
0.062
0.25
Concentration [μM]

FIG. 3

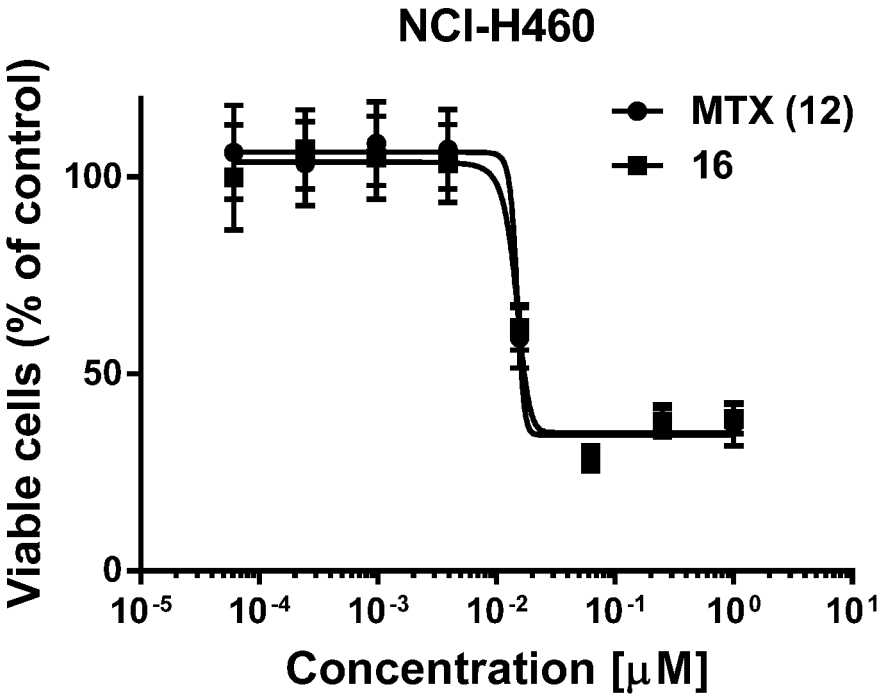
NCI-H460
MTX (12)
16
Viable cells (% of control)
100
50
0
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
Concentration [μM]

FIG. 4

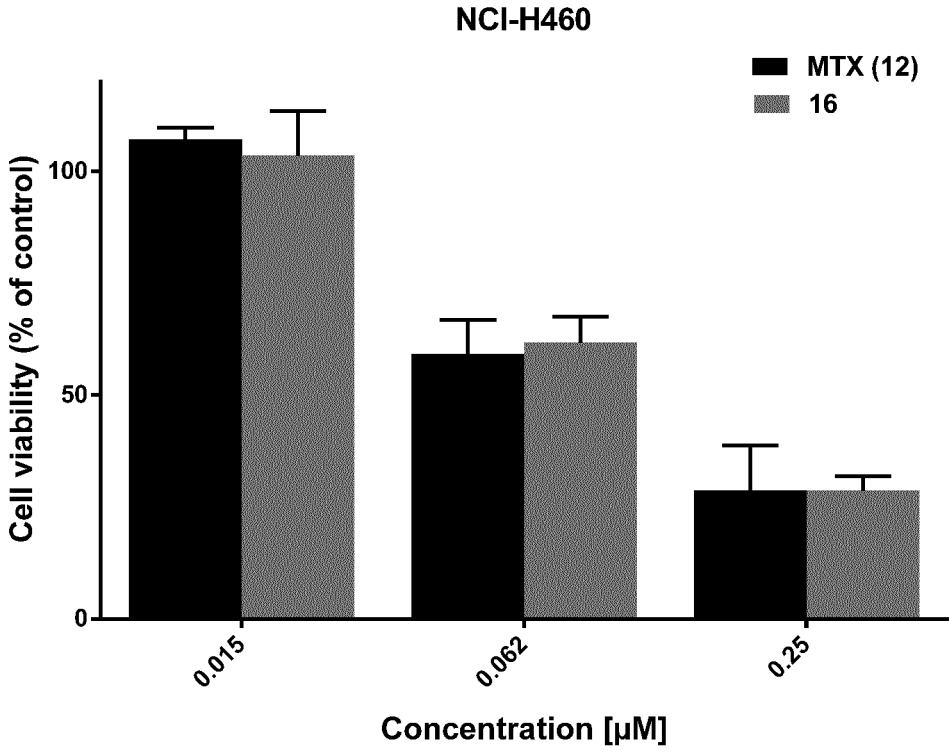
NCI-H460
MTX (12)
16
Cell viability (% of control)
100
50
0
0.015
0.062
0.25
Concentration [μM]

FIG. 5

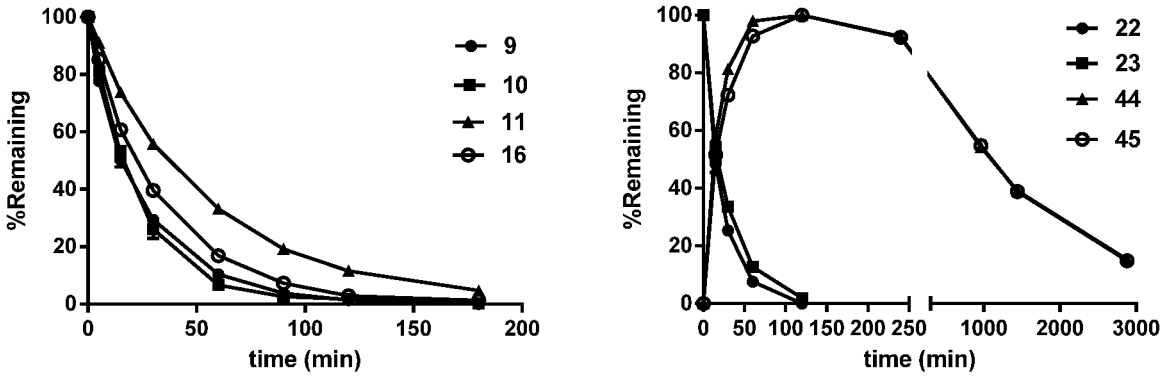

FIG. 6

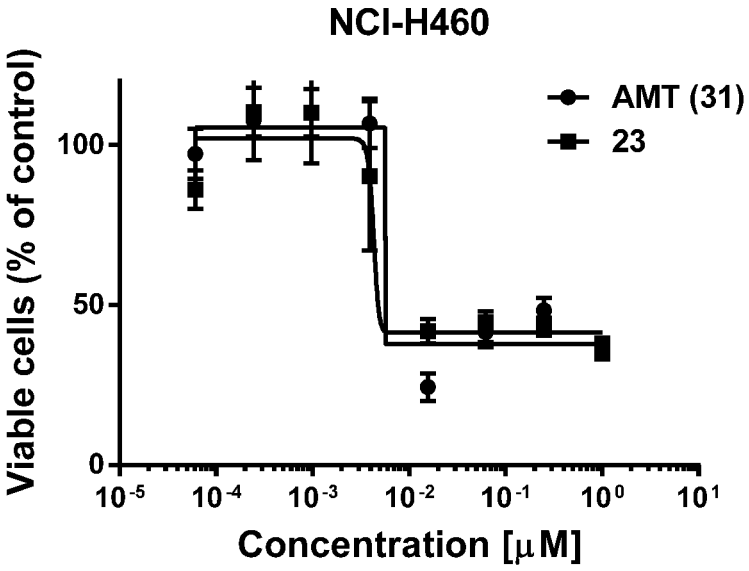
NCI-H460
AMT (31)
23
Viable cells (% of control)
100
50
0
10^{-5} 10^{-4} 10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1}
Concentration [μM]

FIG. 7

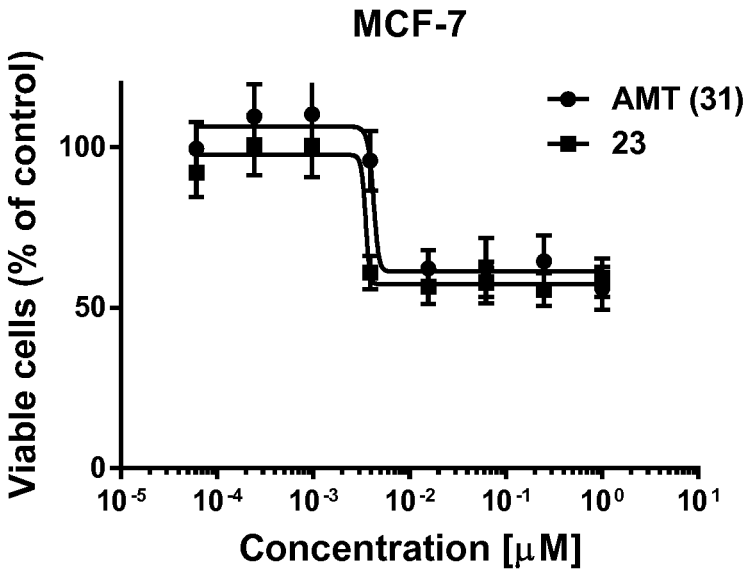
MCF-7
AMT (31)
23
Viable cells (% of control)
100
50
0
10^{-5} 10^{-4} 10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1}
Concentration [μM]

FIG. 8

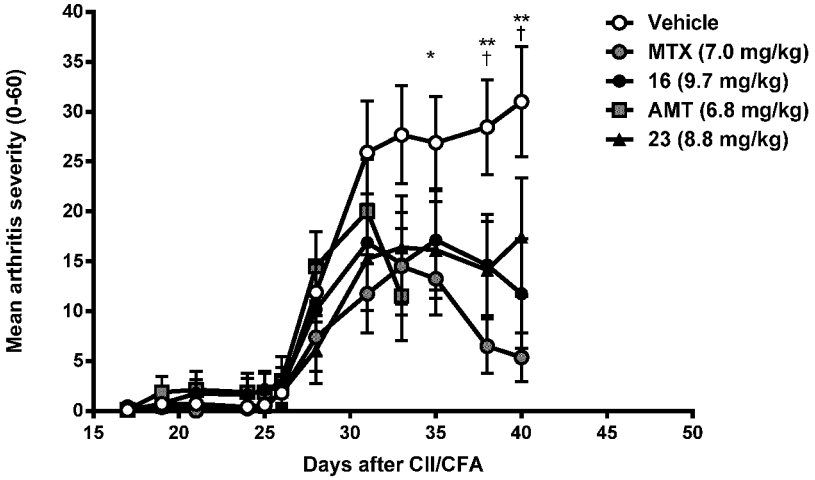
Mean arthritis severity (0-60)
Days after CII/CFA
Vehicle
MTX (7.0 mg/kg)
16 (9.7 mg/kg)
AMT (6.8 mg/kg)
23 (8.8 mg/kg)
*
**
†
**
†

FIG. 9

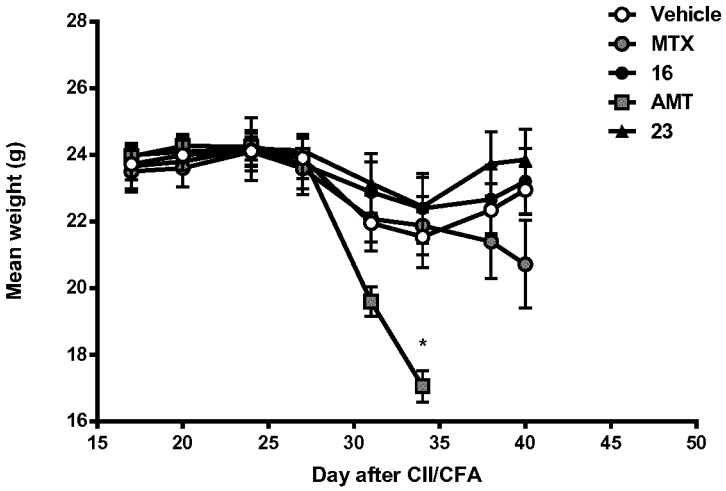
Mean weight (g)
Day after CII/CFA
Vehicle
MTX
16
AMT
23
*

FIG. 10

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/071457

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D487/04 C07F5/02 A61K31/519 A61P29/00 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D C07F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WEI WEN-HAO ET AL: "Gadolinium texaphyrin-methotrexate conjugates. Towards improved cancer chemotherapeutic agents", ORGANIC & BIOMOLECULAR CHEMISTRY, ROYAL SOCIETY OF CHEMISTRY, GB, vol. 3, no. 18, 21 September 2005 (2005-09-21), pages 3290-3296, XP002608176, ISSN: 1477-0520 Scheme 3: compound 8 ----- -/--	1-24

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 October 2017	16/10/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Baston, Eckhard

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ACHILLI, C. ET AL.: "Folic acid-conjugated 4-Amino-Phenylboronate, a Boron-Containing Compound Designed for Boron Neutron Capture Therapy, is an Unexpected Agonist for Human Neutrophils and Platelets", CHEM BIO DRUG DES, vol. 83, 2013, pages 532-540, XP002762878, figure 1	1-24
X	ROSOWSKY A ET AL: "SYNTHESIS AND BIOLOGICAL ACTIVITY OF METHOTREXATE ANALOGUES WITH TWO ACID GROUPS AND A HYDROPHOBIC AROMATIC RINGIN THE SIDE CHAIN", JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY, US, vol. 34, no. 2, 1 January 1991 (1991-01-01), pages 574-579, XP002927775, ISSN: 0022-2623, DOI: 10.1021/JM00106A016 compound 4	1-24
A	KHAN, Z.A. ET AL: "Methotrexate: a detailed review on drug delivery and clinical aspects", EXPERT OPINION ON DRUG DELIVERY, vol. 9, 2012, pages 151-169, XP002762879, the whole document	1-24
A	US 2013/045949 A1 (PENG XIAOHUA [US] ET AL) 21 February 2013 (2013-02-21) the whole document	1-24

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2017/071457

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2013045949 A1	21-02-2013	US 2013045949 A1	21-02-2013
		US 2014200250 A1	17-07-2014